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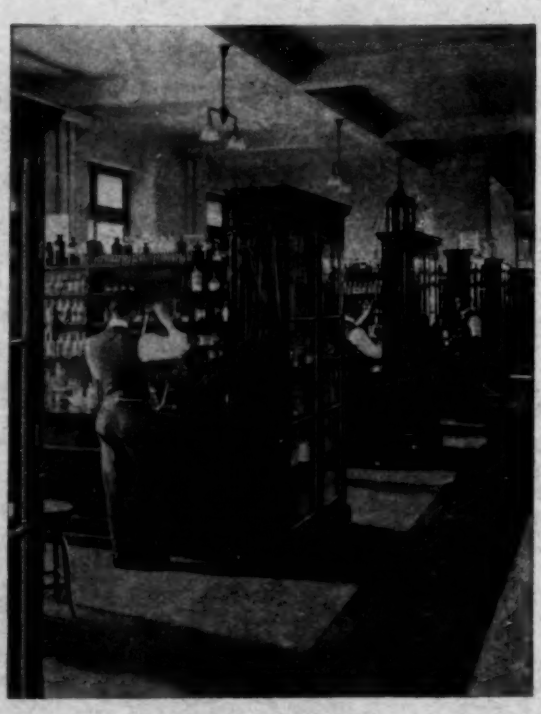

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CLINICAL AND EXPERIMENTAL

BLOOD GROUPS IN GENERAL PARALYSIS

A STUDY OF THE AGGLUTININ-AGGLUTINOGEN FORMULA IN NINETY-ONE CASES*

BY HENRY A. BUNKER, JR., M.D., AND SIDNEY MEYERS, NEW YORK, N. Y.

THERE is a considerable body of evidence at hand that the isoagglutinin content of human blood is a constitutional character. The four isoagglutinin groups traditionally derivable on the basis of the presence or absence of agglutinable substances *a* and *b* (Jansky,[1] Moss[2]) occur as fixed biochemical conditions, with an incidence which varies rather definitely with different races[3] and which is not susceptible to alteration by environmental influences.[4] There is considerable evidence, moreover, that the presence or absence of the isoagglutinable elements *a* and *b* is an hereditary quality, transmitted from parent to offspring.[5] It is interesting that the precise mechanism of this transmission has recently come into dispute: whereas it has long been assumed to involve independent pairs of factors (the agglutinogens *a* and *b* being dominant to their respective isoagglutinins *A* and *B*), Bernstein[6] has recently suggested that the blood groups are inherited as a series of three multiple allelomorphs (the agglutinogens *a* and *b* being both dominant to the same recessive (*R*), thus taking the agglutinins out of hereditary control and leaving only the agglutinogens to be inherited); and Snyder,[3] after an analysis of all the matings so far published, including a particularly extensive series of his own, concludes that the older hypothesis must be discarded.

Whether or not constitutional factors are the basis of the proclivity on the part of a small proportion of individuals previously infected with syphilis to develop parenchymatous syphilitic involvement of the central nervous system (general paralysis) is a question which we are now investigating from certain standpoints. Incidental to this work we have paused to determine

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the isoagglutinin groups to which a series of ninety-one patients suffering from general paralysis belonged, in order to discover whether the incidence of the four blood groups among these patients differed from that of the population at large, or—what is the same thing, as Straszynski[7] has recently shown—from that of syphilities in general. It must be admitted, however, that no correlation between blood group and any constitutional disorder has so far been demonstrated; in no instance thus far recorded has the incidence of the blood groups among the subjects of a given disease differed decisively from that of the general population. Hirszfeld and Brokman,[8] for example, have shown that although susceptibility or immunity to diphtheria is, like the isoagglutinin content of the blood, an inheritable and constitutionally conditioned character, the isoagglutinin "races" nevertheless do not differ in immunity to diphtherial infection: "susceptibility or immunity to diphtheria is not closely connected with either of the biochemical properties of the blood." The absence of any linkage of this character has been demonstrated likewise for syphilis,[7] and by Snyder[3] for dementia precox, epilepsy and mental deficiency; only in the case of cancer is some doubt perhaps permissible, since Weitzner,[9] and still more recently Bendien,[10] have both reported finding Group IV (Moss) represented among carcinomatous subjects to a smaller degree, and Group I to a greater degree, than in normal persons. Hence the possibility that patients with general paralysis might differ from other syphilities in this respect appears rather a remote one, in spite of the moderate degree of correlation which Straszynski[7] has reported to exist between the blood group and the persistence of the Wassermann reaction under treatment. Nevertheless, it seemed of interest to determine the isoagglutinin groups to which the patients in our series belonged, even though in the end this procedure might be found to have afforded only the opportunity to corroborate the findings of certain workers who have demonstrated the apparent existence in human blood of isoagglutinins other than the traditional two (*a* and *b*) first postulated by Landsteiner.[11]

PRELIMINARY CLASSIFICATION

For the carrying out of agglutination tests by the microscopic method, blood was drawn from the vein, the serum pipetted off after standing overnight, and the corpuscles, obtained by mixing about 0.5 c.c. of blood with 1.5 per cent sodium citrate in normal salt solution, centrifuged and washed three times with normal saline solution. Hanging-drop preparations were then set up in duplicate in the usual manner, using two loopfuls of serum and one loopful of a 1 per cent suspension of corpuscles in salt solution. The preparations were examined at the time, and at the end of one and two hours, and in the case of negatives at the end of four hours as well; during these intervals they were kept in the ice box at 5° to 10° C., on account of the fact, well brought out by Guthrie and Pessel,[12] that an amount of agglutinin sufficient to cause marked agglutination in the ice box may escape recognition in tests made at 37° C. or at room temperature. Autoagglutination (said to be a rare phenomenon in human bloods), which takes place only at low temperatures and might accordingly lead to confusion, was readily excluded by a

preliminary matching of the serum of each patient against his own corpuscles; only two instances of autoagglutination in the series were discovered; in each the autoagglutinin was removed from the serum, prior to its further use, by absorption with the corpuscles of the same individual.

All tests were performed by matching both the unknown serum and the unknown corpuscles against known corpuscles and known serum, respectively, in accordance with the schema shown in Table I.

TABLE I

SERUM	x RED CELLS			
Group II	+	0	+	0
Group III	+	+	0	0
Assigns x to	Group I	Group II	Group III	Group IV*

Serum	Red Cells		Assigns x to
	Group II	Group III	
x	0	0	Group I
x	0	+	Group II
x	+	0	Group III
x	+	+	Group IV

*The group-numbering of Moss is here employed, rather than the now official nomenclature of Jansky, for the sake of uniformity with Guthrie, upon whose work the present investigation is based.

The results, thus checked against each other, are presented in Table II, which gives the distribution of the four traditional blood groups among the ninety-one general paralytics in this series, as compared with their distribution among the population at large.

TABLE II

	91 PATIENTS WITH GENERAL PARALYSIS*	GENERAL POPULATION		
		3,000 EUROPEANS (HIRSZFELD[3])	1,122 AMERICANS U. S. ARMY (MOFFITT[3])	5,000 AMERICANS (CULPEPPER[3])
Group I	4.5	4.5	4.8	5.2
Group II	41.5	41.4	53.6	36.0
Group III	14.5	11.3	6.2	14.3
Group IV	39.5	42.8	35.4	44.5

*From the racial standpoint it might be mentioned that our material was made up of 21 Jews (5 American-born) and 70 Gentiles (45 American-born); all were Caucasians except four negroes, two of whom were members of Group III.

It is thus apparent that the patients in this series do not differ essentially from the general population with reference to the percentage claimed by each of the four blood groups. General paralysis, then, no more than syphilis itself,[7] is characterized by any difference from the population at large in the incidence of the blood groups which it exhibits.

GROUP II CASES

In an admirable series of papers which we may warmly recommend to the reader as a most able consideration of the entire subject, Guthrie and Huck[13] have pointed out that "the methods generally employed for blood grouping are based upon the assumption that there are only four isoagglutinin groups, and that the blood of every human being belongs in one

of these groups. The tests based on this assumption," they go on to say, "are so devised as to cause each blood tested to fall into one or other of these four groups, thus serving to perpetuate a belief which no one has seriously questioned." They then present evidence that "the popular belief concerning the existence of four and only four isoagglutinin groups is incorrect" through the demonstration, by direct tests and by absorption experiments, of the existence of at least a third isoagglutinin and a third isoagglutinogen.

Without following out each step which marked the pioneer work in this direction of Guthrie and his coworkers, we may summarize their results, first, as obtained among the members of "Group II."

They found that, in the first place, the erythrocytes of their various "Group II" individuals did not behave alike. For, briefly stated, Group IV serum, after absorption with the cells of a Group I individual, no longer agglutinated the cells of Group I, Group III, and certain of the members of "Group II," *but did agglutinate the cells of other members* of "Group II"; and when absorbed with the cells of the first-mentioned members of "Group II," lost (naturally) the power to agglutinate the cells of these individuals, *but still strongly agglutinated the cells of the other members of "Group II"* (as well as the cells of Group I and Group III).

TABLE III

SERUM	SUBSEQUENT AGGLUTINATION OF RED CELLS			
	GROUP I	GROUP III	GROUP II*a*	GROUP II*β*
1. Group IV	+	+	+	+
2. Group IV (after absorption with cells of Group I)	0	0	0	+
3. Group IV (after absorption with cells of Group II*a*)	+	+	0	+

Hence there may be assumed the existence of an agglutinogen (*b*) common to both types of "Group II" individuals (Table III: 1. vs. 2. and 3.), and likewise an additional agglutinogen (*c*) present in the erythrocytes of Group II *β* but not in those of Group II *a* (Table III: 2. and 3.). This necessitates, obviously, the presence of the corresponding agglutinin (*C*) in the serum of Group IV.*

The phenomena represented in Table III may be schematized as follows:

Table III: 1. **ABC**† – cross agglutinated with (gr. IV serum)

- **– ab** (gr. I cells) = +
- **– a** (gr. III cells) = +
- **– b** (gr. II *a* cells) = +
- **– bc** (gr. II *β* cells) = +

*Since Group IV serum agglutinated the cells of Group II and Group III (Table III:1.), this serum must contain the agglutinins (*A* and *B*) corresponding to the agglutinogen (*b*) present in the cells of Group II and to the agglutinogen (*a*) present in the cells of Group III. For it will be remembered that Group II and Group III are reciprocally agglutinative, and the sera of both agglutinate the corpuscles of Group I (Table 1). Hence we assume, in the accepted manner, the presence in the *cells* of Group III of an agglutinogen (*a*) corresponding to the agglutinin (*A*) in the *serum* of Group II, and in the *serum* of Group III an agglutinin (*B*) corresponding to the agglutinogen (*b*) in the *cells* of Group II; therefore in the cells of Group I both the agglutinogens (*a* and *b*) possessed by the cells of Group II (*b*) and Group III (*a*).

†For convenience the elements which take part in absorption or agglutination are designated in heavy-faced type.

Table III: 2. **ABC** – (gr. IV serum) absorbed with – ab (gr. I cells) ⟶ C – (absorbed serum)

C – (absorbed serum) cross-agglutinated with
- – ab (gr. I cells) = 0
- – a (gr. III cells) = 0
- – b (gr. II *a* cells) = 0
- – bc (gr. II *β* cells) = +

Table III: 3. **ABC** – (gr. IV serum) absorbed with – b (gr. II *a* cells) ⟶ AC – (absorbed serum)

AC – (absorbed serum) cross-agglutinated with
- – ab (gr. I cells) = +
- – a (gr. III cells) = +
- – b (gr. II *a* cells) = 0
- – bc (gr. II *β* cells) = +

It therefore follows that Group IV serum, from which agglutinin *B* has been removed by absorption with Group II *a* cells, will thus serve to distinguish between the *a* and *β* members of "Group II"; for agglutination of the II *a* cells, which contain only agglutinogen *b*, will not be affected by a serum lacking agglutinin *B*, whereas agglutination of the II *β* cells, which contain agglutinogen *c* as well as *b*, will be readily brought about through the presence in the absorbed serum of agglutinin *C*.

It was accordingly a simple matter to follow the procedure outlined by Guthrie and Huck,[13] of absorbing serum from successive members of Group IV with erythrocytes from successive members of "Group II" until a serum was found which would completely agglutinate the corpuscles of an individual apparently belonging to Group II, after that serum had been entirely deprived of agglutinins for the corpuscles of other individuals classified in the same group.* Having found such a group IV serum, it was needful only to perform cross-agglutination tests with the corpuscles of all the "Group II" patients in our series to determine which of them had the agglutinogen formula *–b* and which the formula *–bc*.

In this way it was discovered that of 37 patients in "Group II," 29 possessed the additional agglutinogen *c* and 8 were without it; that is, 78 per cent of the group contained the extra agglutinogen in their corpuscles.

Guthrie and Huck[13] expressed themselves only tentatively at first with regard to the relative incidence of these two formulae within "Group II," believing the formula *–b* to be commoner than *–bc*. Later, however, they were led to question the accuracy of their earlier impression concerning the relative frequency with which these two types are encountered within "Group II."[14] Within the year, Coca and Klein,[15] furthermore, described an additional agglutinable substance which they termed *X* and which they believed to be

*In performing the absorption experiments we found it often advisable to employ a larger volume of corpuscles in proportion to serum than did Guthrie and Huck—about 10 volumes of packed corpuscles (a 75 per cent suspension) to 15 volumes of serum, rather than 9 volumes of the former to 20 volumes of the latter. Like these writers we carried out all our absorptions in the ice box, but often for four to six hours rather than for two hours.

possibly identical with the agglutinogen *c* of Guthrie and Huck; and they found it in about 75 per cent (15 out of 23) of their "Group II" bloods. More recently Kline and his coworkers[16] found this extra agglutinogen, which they proved was identical with the agglutinogen *c* of Guthrie and Huck, in 81 per cent of two hundred "Group II" individuals.[17] So that once again, this time with respect to the agglutinogen formula of "Group II" bloods, our general paralytics appear to present no difference from the usual.

Following up the further work of Guthrie, Pessel and Huck,[18] we then absorbed the serum of each "Group II" individual in our series with the corpuscles of a member of Group I; the absorbed serum was then cross-agglutinated with the corpuscles of the same Group I individual and with those of five members of Group III. As is evident from Table IV, these various "Group II" sera did not behave alike, in that some of them, after absorption, continued to agglutinate Group III corpuscles as before, while others failed to do so.

TABLE IV

SERUM	ABSORBED WITH R.B.C.	GR. I (I.K.)	GR. III (S.J.)	GR. III (J.H.)	GR. III (A.R.)	GR. III (H.B.)	GR. III (G.K.)
1. Group II (S.M.).	Group I (I.K.)	0	+	+	+	+	+
2. Group II (L.D.)	Group I (I.K.)	0	+	+	+	+	+
3. Group II (M.H.)	Group I (I.K.)	0	+	+	+	+	+
4. Group II (M.I.)	Group I (I.K.)	0	+	+	+	+	+
5. Group II (J.H.)	Group I (I.K.)	0	0	0	0	0	0
6. Group II (F.G.)	Group I (I.K.)	0	0	0	0	0	0
7. Group II (T.K.)	Group I (I.K.)	0	0	0	0	0	0
8. Group II (H.R.)	Group I (I.K.)	0	0	0	0	0	0

Since this Table IV is similar, on a reduced scale, to that published by Guthrie *et al.*,[18] we may quote their own words in this connection: "There was evidently an agglutinin (*D*) present in the serum of "Group II" [S.M.], "Group II" [L.D.], "Group II" [M.H.], and "Group II" [M.I.], which was not present in the serum of "Group II" [J.H.], "Group II" [F.G.], "Group II" [T.K.], and "Group II" [H.R.]. It is also plain that the corresponding agglutinogen (*d*) was present in the erythrocytes of the five members of Group III, but absent from those of Group I." Now since each member of "Group II" agglutinated the corpuscles of all five Group III bloods, the two agglutinogens in the latter might have reacted either (1) with agglutinin *A* in patient J.H. and with *D* in patient S.M., or (2) with *A* in patient J.H. and with *AD* in patient S.M. But as Guthrie points out, each of the Group II sera agglutinated the cells of Group I, though it is evident that the latter are devoid of agglutinogen *d* (Table IV); hence there must be some agglutinin other than *D* in *all* the "Group II" sera; therefore agglutinin *A* is present in all the "Group II" sera, and agglutinin D is present in addition in some of them.[18]

We may schematize the foregoing as follows:

Table IV: 1. **AD**– absorbed with –**abc** ⟶ **D**–
(gr. II (S. M.) serum) (gr. I (I. K.) cells) (absorbed serum)

D– cross-agglutinated with –**ad** = +
(absorbed serum) (gr. III cells)

Table IV: 5. A – (gr. II (J. H.) serum) absorbed with –abc (gr. I (I. K.) cells) ⟶ O – (absorbed serum)

O – (absorbed serum) cross-agglutinated with –ad (gr. III cells) = 0

By combining the results of this experiment with those of the previous one, we find that the "Group II" individuals in our series are represented by the agglutinin-agglutinogen formulae above discussed in the following proportions.

TABLE V

AD-bc	27	32 containing *D* (86 per cent)
AD-b	5	
A-bc	2	5 not containing *D*
A-b	3	

Guthrie, Pessel and Huck[18] state that they lack present information concerning the relative frequency of these types of "Group II" bloods; neither are we justified in drawing conclusions on this point on the basis of the few cases here reported, although it would seem that the presence of the extra agglutinin *D* and of the extra agglutinogen *c* is far commoner than the absence of either. On the other hand, these observers did not happen to encounter a type possessing neither the extra agglutinin nor the extra agglutinogen (i.e., formula *A-b*), though they speak of its probable existence. In the present series, however, we found three bloods (verified by repeated subsequent examinations) which lacked both agglutinin *D* and agglutinogen *c*. Our experience also differs from that of Guthrie to the extent that we were able to find but two examples of the absence of agglutinin *D* with the presence of agglutinogen *c* (formula *A-bc*). The three types of "Group II" blood discovered and described by Guthrie and his coworkers[18] occurred, then, in our series of 37 "Group II" general paralytics in the proportions of 86 per cent. (*AD-bc*), 7 per cent (*AD-b*), and 3 per cent (*A-bc*), respectively; the fourth possible type (*A-b*), which Guthrie did not happen to encounter, made up 4 per cent of the group.

GROUP III CASES

The twelve Group III individuals in this series (one patient died before the further tests could be carried out) may be discussed very briefly.

It is clear that the two types of "Group II" blood which lack or possess agglutinogen *c* may be used to demonstrate the presence of agglutinins *B* and *C*. If a Group III serum be matched against a "Group II" blood of the formula *–b*, agglutination will of course occur (Table I): the serum contains agglutinin *B*. If this agglutinin is now removed by absorption of the serum with the corpuscles of a "Group II" blood of formula *–b*, and the absorbed serum then matched against the corpuscles of a "Group II" blood of formula *–bc*, the occurrence of agglutination must necessarily indicate the presence in the absorbed serum of agglutinin *C*.

$$\underset{\text{(gr. III serum)}}{\mathbf{BC}-} \xrightarrow{\text{absorbed with } -\mathbf{b}\ (\text{gr. II } \alpha \text{ cells})} \underset{\text{(absorbed serum)}}{\mathbf{C}-}$$

$$\underset{\text{(absorbed serum)}}{\mathbf{C}-} \xrightarrow{\text{cross-agglutinated with } -\mathbf{bc}\ (\text{gr. II } \beta \text{ cells})} = +$$

Accordingly, we absorbed each of the twelve Group III sera in our series with the corpuscles of a "Group II" blood of known *–b* formula, and then matched these absorbed sera, from which agglutinin *B* had thus been removed, against the corpuscles of a "Group II" blood of known *–bc* formula. Strong agglutination took place in every instance, thus demonstrating the presence in each of the twelve Group III sera of agglutinin *C* as well as of agglutinin *B* (Table VI).

TABLE VI

SERUM	ABSORBED WITH R.B.C.	SUBSEQUENT AGGLUTINATION OF R.B.C. GR. II (*–b*) (J.R.)	GR. II (*–b*) (J.H.)	GR. II (*–bc*) (G.H.)
Group III (J.H.)	Group II (J.H.)	0	0	+
Group III (A.R.)	Group II (J.H.)	0	0	+
Group III (J.S.)	Group II (J.H.)	0	0	+
Group III (G.K.)	Group II (J.H.)	0	0	+
Group III (S.J.)	Group II (J.H.)	0	0	+
Group III (H.B.)	Group II (J.H.)	0	0	+
Group III (D.S.)	Group II (J.H.)	0	0	+
Group III (L.S.)	Group II (J.H.)	0	0	+
Group III (B.D.)	Group II (J.H.)	0	0	+
Group III (D.B.)	Group II (J.H.)	0	0	+
Group III (C.C.)	Group II (J.H.)	0	0	+
Group III (V.D.)	Group II (J.H.)	0	0	+

It is equally clear that if the serum of a "Group II" blood of known formula *AD–* be absorbed with the cells of a Group I blood (I.K.), agglutinin *A* will be removed from the "Group II" serum and agglutinin *D* remain; hence this absorbed serum, containing only agglutinin *D*, may be used to detect the presence or absence of agglutinogen *d*. Accordingly, the serum of a "Group II" patient (G.H.; formula *AD-bc*) was absorbed with the corpuscles of a Group I patient (I.K.; formula *O-abc*), and the absorbed serum matched against the cells of each of the twelve Group III bloods in our series. Agglutination took place in every instance, thus demonstrating the presence in the corpuscles of each of the twelve Group III cases of agglutinogen *d*, as well as of agglutinogen *a* (since the same corpuscles are also agglutinated by "Group II" serum not containing agglutinin *D*).

This uniform agglutinogen formula *–ad* for the corpuscles of Group III individuals is in accord with the observations of Guthrie and Pessel,[19] for they found agglutinogen *d* associated with agglutinogen *a* in the erythrocytes of seven members of this group. The agglutinin-agglutinogen formula for the twelve Group III patients in this series thus appears to be *BC-ad*.

GROUP I CASES

It has already been seen that Group IV (J.W.) serum, after absorption with "Group II" (*–b*) corpuscles, was still able to agglutinate "Group II" (*–bc*) corpuscles, by virtue of possessing agglutinin *C* as well as agglutinin *A*.

If now from this absorbed serum agglutinin *A* is also removed by absorption with erythrocytes belonging to a Group III individual, this serum will contain only agglutinin *C*, and may therefore be used for the detection of agglutinogen *c*. Accordingly the serum of J.W., twice absorbed in this way, was matched against the corpuscles of the four Group I patients in our series. In each instance strong agglutination took place, demonstrating the presence in each of the four of agglutinogen *c* as well as of agglutinogens *a* and *b*.*

The agglutinin-agglutinogen formula of all four of our Group I patients thus appears to be *O-abc*. On the other hand, Guthrie and his coworkers[13, 19] found two Group I cases with the formula *O-ab* and three with the formula *O-abc*. It seems possible that the latter is the commoner.

THE WASSERMANN REACTION

Straszynski[7] has recently demonstrated a certain degree of correlation between blood group and the readiness with which the Wassermann reaction disappears after antisyphilitic treatment. Having divided a series of 325 patients into 209 in whom the Wassermann became negative after only one or two courses of arsphenamine, and 116 whose Wassermann remained positive in spite of more prolonged treatment, he observed that to the latter category belonged 22 per cent of the 95 Group IV (Moss) individuals in the series, 36 per cent of the 119 Group II patients, 44 per cent of the 81 Group III patients, and 53 per cent of the 30 Group I patients. Our cases are hardly comparable to Straszynski's, not only because of being general paralytics, who are notoriously Wassermann-fast, but because the treatment which they received—malaria or tryparsamide or both—is scarcely calculated to bring about Wassermann negativity in the blood. Of the 71 patients in the present series observed for more than one year (some of them for periods up to three years in length), 70 per cent showed little or no change in the blood Wassermann; in 10 per cent the strength of the reaction had become definitely modified; in 20 per cent it had become essentially negative. When considered in relation to the blood group, as shown in Table VII, it is seen that the Wassermann reaction in the serum remained practically unchanged in 61 per

TABLE VII

WASSERMANN REACTION IN THE BLOOD	GROUP II NUMBER PER CENT	GROUP III NUMBER PER CENT	GROUP IV NUMBER PER CENT
Unchanged ++ to ++++ alc. antigen +++ to ++++ chol. antigen	17–*61*	10–*91*	21–*75*
Definitely Modified Neg. to + alc. antigen ++ to ++++ chol. antigen	4–*14*	0	2–*7*
Negative Neg. alc. antigen Neg. to ++ chol. antigen	7–*25*	1–*9*	5–*18*
	28	11	28

*For Group I (I.K.) corpuscles lack agglutinogen *d* (Table IV), and absorption of Group III serum by Group I corpuscles leaves no agglutinin in the Group III serum for any of the four groups,[19] *both B* and *C* being removed by the Group I corpuscles employed in the absorption.

cent of the 28 Group II patients, in 75 per cent of the 28 Group IV patients, and in 91 per cent of the 11 Group III patients; and became negative in 9 per cent of the Group III cases, in 18 per cent of the Group IV cases, and in 25 per cent of the Group II cases. No correlation could be discovered between the behavior of the Wassermann reaction and the actual agglutinin-agglutinogen formula of the "Group II" individuals.

Disregarding Group I, of which the members in our series are too few for inclusion, and making allowance for the small number of observations reported, our results are in accord with those of Straszynski (even though he found Group IV least Wassermann-fast, as compared with Group II in our material) at least to the extent that Groups II and IV, taken together, appear to present some contrast to Groups I and III with reference to the disappearance of the Wassermann reaction; but whether this suggests a correlation between the behavior of the Wassermann reaction in this respect and the presence of agglutinin *A*, since this is common to Groups II and IV and absent from both Groups I and III, it would naturally be hazardous to say. In any case, we hardly feel that our results offer anything in the way of comment upon those of Straszynski; indeed, considering the quantitative and qualitative limitations of our data, we are led to mention them only because it appears to us to be of some interest, in the light of his observations, that the few patients who exhibited any tendency to Wassermann-negativity under the nonspirochetocidal type of therapy employed should nearly all have belonged to Groups II and IV, especially perhaps the former.

SUMMARY

1. The incidence of the traditional four isoagglutinin groups was found to be the same in a series of 91 patients with general paralysis as in the population at large. Since this is also true of syphilities, general paralytics do not differ from syphilities in general in this respect.

2. An extra agglutinogen (*c*) in the corpuscles of "Group II" individuals, described by Guthrie and his coworkers and later by others, was found in the present series in the same proportion of "Group II" bloods (about 80 per cent) as reported by others.

3. An extra agglutinin (*D*) in the serum of "Group II" individuals, first described by Guthrie, was found in 32 out of 37 members of "Group II" in the present series (86 per cent).

4. Our patients, being general paralytics who received treatment only in the form of malaria or tryparsamide or both, showed little tendency to definite modification or negativity of the Wassermann reaction in the blood; all but two of the twenty patients who exhibited such a tendency were members either of Group II or Group IV (Moss), eleven of the latter eighteen belonging to Group II.*

*Since the foregoing article was submitted for publication, there has appeared in the Ztschr. f. d. ges. Neurol. u. Psychiat. of November, 1926, a report of blood-group determinations carried out on a group of 100 general paralytics by Jacobsohn, who found among these cases 3 per cent of group I, 47 per cent of group II, 17 per cent of group III, and 33 per cent of group IV individuals—essentially the same proportions as in our own series.

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MESENTERIC ENTEROCYSTOMA*

By Wilhelm Hueper, M.D., Chicago, Ill.

CYSTIC tumors in the mesentery are relatively rare. The majority of cystic formations located in the mesentery are not genuine neoplasms, but are cysts of various origins as parasitic or bacterial infection (ecchinococcus cysts, cysts of the cysticercus cellulosae, gas cysts known as pneumatosis cystoides intestini); or due to trauma of the abdomen causing a localized hemorrhage or lymphorrhagia; central necrosis and liquefaction of infected lymph glands (tuberculosis, typhoid fever) or of solid tumors (lipomas). Among the true tumors of the mesentery the cystic new growths exceed by far in number the solid neoplasms (ratio 4:1 according to Martin[1]). After being formerly classified according to their content (blood, lymph, chylous, caseous material) the mesenteric cysts are grouped at present according to their origin and histologic structure, because the content is not a sufficient criterion of the actual character of the tumor.

Classification:

1. Cystic lymphangiomas.
   a. Serous cysts.
   b. Chylous cysts.
2. Enterocystomas.
3. Cysts being derived from the wolffian duct.
4. Dermoid cysts.
5. Teratomas.
6. Fetal inclusions.
7. Teratoid mixed tumors.

FREQUENCY

The cystic lymphangiomas rank highest in number with more than 200 cases reported (Forster[2]). The frequency in the other groups is much lower: enterocystomas 27, cysts being derived from the wolffian duct 5, dermoid cysts 33, teratomas 8, fetal inclusions 9 and teratoid mixed tumors 2 (Dowd,[3] Niosi,[4] Colmers,[5] Sommer[6]).

On account of the rarity of the mesenteric enterocystomas and with reference to the pending discussion of their origin we wish to report a new case of mesenteric enterocystoma which came recently to our observation.

REPORT OF CASE

History.—B. S., boy aged 5, was admitted to Mercy Hospital in May, 1925, previous history negative, except tuberculous lymph glands on the neck in the last two years. Three days prior to admission, the patient became suddenly sick with pains in the stomach region,

*From the Department of Pathology, Loyola University, School of Medicine, Chicago, Ill.

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lasting until the next morning. The patient was feeling well till in the evening the pains started again with increased strength, but localized more around the navel and combined with vomiting during the night. The pains subsided again in the morning of the third day having a new onset in the afternoon, when the child was first seen by Dr. I. F. Golden, to whom I am greatly indebted for the clinical history of the case. The abdomen was slightly distended at that time, the right hypogastric region was tender and showed muscular rigidity. A hen-egg sized, movable tumor of an elastic consistency and of a smooth surface was felt in the right hypogastric region near the navel. Leucocyte count 22,400. *Diagnosis:* Acute appendicitis and mesenteric cyst. A hen-egg sized cyst located bewteen the two membranes of the mesentery, 2½ inches from the ileocecal valve adjacent, but not connected with the ileum was enucleated at operation. Also appendectomy was performed.

Histologic examination.—The wall of the cyst was about 0.2 cm. thick, showing on the rather smooth inner side a small nodule, rice-sced size, projecting into the lumen. Microscopically the wall was composed of five layers: serosa, subscrosa, muscularis, submucosa and mucosa. Serosa and muscularis showed a similar structure to that found in the corresponding layers of the normal intestinal wall. The submucosa was a rather dense connective tissue and thinner than the same layer in the normal intestinal wall. The mucosa was formed by a single layer of cubical or low cylindrical epithelial cells. Goblet cells were not observed. Rudimentary villi were present in a small area. Glandular formations, resembling in location and structure Brunner's glands were seen in some places. In other parts of the wall the epithelial lining was completely lacking. Lymphoid tissue was only found in the above-mentioned nodule. The appendix showed a moderate hyperplasia of the lymph follicles. There were no signs of an acute inflammation.

DISCUSSION

The mucosa and submucosa of the cyst just described represent the type of intestinal structure which is present in an early stage of fetal life. At that time the intestinal mucosa is composed of a single layer of cylindrical cells and does not show any villi. Also the lymphoid tissue in the submucosa is not yet developed in this stage of prenatal development. In other cases a complete imitation of a fully developed intestinal mucosa with villi and glandulae Lieberkuhni was reported. But irregularities and defects in the development of individual layers were frequently seen, as lack of the lymphoid tissue or of one layer of the muscularis or as partial or complete fibrosis of the muscularis. Structural imperfections of the last-mentioned kind will naturally enhance greatly the difficulty of an exact interpretation. But in discarding those tumors from this group only the complete lack of muscular tissue may justify such a step. Investigations of Schmitt[7] have shown that sometimes very small rests of muscular tissue may be present in an entirely fibrotic middle layer.

ORIGIN

The majority of the enterocystomas originate from rests of the omphalomesenteric duct. The most common malformation resulting from an incomplete obliteration of this duct on its intestinal end is the Meckel's diverticulum, which is found in 2 per cent of men. In other cases the duct remains open through its whole length or only in its distal or central part. Rests of this kind may gain a certain autonomy and cystic tumors of the structure and character of enterocystomas are the result. They are found in accordance with the location of the omphalomesenteric duct, in the abdominal wall near

the navel, in the mesentery, and on the convex side of the intestine (Colmers[5]). From 43 enterocystomas 4 were in the abdominal wall, 25 on the convexity of the intestine and 15 on the concave side of the intestine or in the mesentery. Corresponding with the usual location of Meckel's diverticulum 80 cm. above the ileocecal valve on the ileum the enterocystomas are seen most frequently in this region of the ileum or in the adjacent mesentery. But the great variation in the location of the diverticulum, which is found on the small intestine from the duodenum to the ileocecal valve explains also the occurrence of enterocystomas along this line either connected with the bowel or free in the mesentery.

But evidently not all enterocystomas are derived from rests of the omphalomesenteric duct. There are 2 cases reported in which multiple cysts were observed. Roth[8] described the occurrence of many small cysts along the ileum in the mesentery and Schmincke[9] saw one cyst in the mesentery and a second one in the dorsal mediastinum. The peculiar location of the multiple cysts in these two cases is an absolute argument against their origin from rests of the omphalomesenteric duct. The source of these cysts was presumably displaced embryonal intestinal tissue. Observations of Elze[10] on the fetal intestine, where he found epithelial formations interpreted by Aschoff[11] as processes of epithelial cells into the intestinal wall, may offer some substantiation of this conception if we consider that these processes may become displaced to the outside of the intestine.

Also during my own investigations on the duodenum of thirty rats and rabbits I found in the wall of the duodenum of one rabbit a small cyst located in the subserosa. The cyst had a well-developed muscularis and a mucosa which was similar to that of the duodenum only thinner. We are more inclined to interpret this cyst as a malformation of the intestinal wall on account of its close relation to it than as a rest of the omphalomesenteric duct. If the negative results of Woyciechowski who examined without success the mesentery of 48 human bodies for displaced intestinal tissue, seem to disagree with our conception, we have to consider the fact that the small number of cases examined does not allow any definite conclusions in this direction.[12] Furthermore we do not believe that enterocystomas may be produced by the traction of displaced pancreatic tissue on the intestinal wall causing diverticula (Hansson[13]), because in none of the cases reported was pancreatic tissue found in the wall of the cysts.

The enterocystomas are generally of minor size. They rarely exceed the size of an apple, although larger ones were observed. Usually there is only one cyst. Rarely two are present connected with each other by a duct. Connections of the lumen of the cyst with the lumen of the intestine through a duct have been described, especially in cases which the cyst was adherent to the wall of the intestine. Malignant degeneration in the epithelial part of the cyst was found in one case (Schmitt[7]), in which a carcinomatous growth was present in nodular formations projecting into the cystic lumen.

CONCLUSIONS

1. A case of mesenteric enterocystoma is reported.

2. Enterocystomas may originate from rests of the omphalomesenteric duct or from displaced embryonal intestinal tissue.

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THE USE OF CHOLIN IN PARALYTIC ILEUS*

By Hans Hartman, M.D., and Wm. Dock, M.D., San Francisco, Calif.

IN 1925 Klee and Grossman[1] reported encouraging results from the administration of cholin intravenously in 120 cases of paralytic ileus. At the same time Magnus[2] summarized the experimental work which had been done in his laboratory on cholin in its relation to the gastrointestinal tract. Cholin is normally present in the muscularis of the stomach, and the large and small bowels. It diffuses out of fresh strips of gut and the fluid thus obtained reestablishes contraction in old intestinal strips from which the cholin has disappeared. While cholin is not absent in the gut in cases of ileus, the intravenous administration causes a return of normal peristalsis in experimental ileus due to infection, prolonged anesthesia, or trauma. The single lethal dose in animals was found to be 35 mg. per kilogram. Over a period of one hour 50 mg. per kilogram could be given with safety. The dose suggested for man was 600 mg. per 60 kilograms given slowly (seventeen minutes). Cholin (trimethyloxyethyl ammonium hydroxide) is given as the hydrochloride, and the crystals or solution should be kept in ampules to prevent decomposition to more toxic substances.

Wolf and Canney[3] in 1926 reported a series of four cases in three of which cholin was successfully used; in the fourth the administration had been too long delayed and there was no effect. Six hundred mg. diluted in 180 c.c. of sterile normal saline solution was given intravenously over a period of time not shorter than seventeen minutes.

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*From the Departments of Surgery and Medicine Stanford University Medical School, San Francisco, Calif.

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As we were unable at the time to obtain cholin from the firm which supplied the above workers we accordingly felt it necessary to standardize the American product (Eastman Kodak Company) as to toxicity. Rabbits were used for this purpose and it was found that 50 mg. per kilogram in a single dose was well tolerated. The lethal dose was 75 mg. per kilogram given in a single dose. The therapeutic dose produced marked salivation, redness of the ears, contraction of the pupils, slowing of the pulse, and peristaltic action even in anesthetized animals in which the gut had been traumatized until ileus resulted.

We have had an opportunity to use this drug in but a single case of ileus, but the immediate result was so striking that further trial seems justified.

An obese woman, forty years old, developed abdominal distention and vomiting following hysterectomy. Gastric lavage, stupes and enemas given over a period of four days failed to relieve the condition; she had no bowel movements or passage of gas. Her temperature rose to 103°, and vomiting of fecal material occurred. No peristalic sounds could be heard. The diagnosis was paralytic ileus, possibly with peritonitis. She was given 0.6 gm. of cholin hydrochloride in 250 c.c. of normal saline solution over a period of twenty minutes. The blood pressure fell from 120 to 80 the first three minutes and there was flushing of the face, salivation, but no appreciable slowing of the pulse rate. Immediately after the intravenous administration had been completed peristalsis was heard and the patient expressed a desire to defecate and in about five minutes passed a large quantity of gas and liquid feces. There were no cramps. The temperature continued to rise and the patient died in twelve hours. At necropsy a partial obstruction without gangrene was found high in the jejunum, which had herniated into the abdominal wound.

CONCLUSION

The use of cholin intravenously in paralytic ileus is justified on both a clinical and experimental basis. The action, both therapeutic and toxic is very fleeting; the rate of administration should be controlled by following the blood pressure. One-half to 1 gm. can be given in fifteen to thirty minutes and repeated if necessary at two to three hours intervals. The cholin should be kept in sealed ampules containing the approximate dose either in crystals or solution as the decomposition products are quite toxic.

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SUGAR IN BLOOD*

A Study of the Accuracy of the Kramer-Gittleman Modification of the Method of Folin and Wu

By Elsa R. Orent, B.S., Brooklyn, N. Y.

A SURVEY of the literature reveals a surprisingly large number of methods for the estimation of sugar in blood. Most of these require quantities of blood such as can only be obtained by entering a vein. A few,[1] of which the Bang[2] method is probably the most widely used, require such quantities of blood as can be obtained by pricking the finger. Such a method has recently been described by Kramer and Gittleman.[3] In their technic, the blood is collected in a small pipette, $\frac{1}{20}$ to $\frac{1}{10}$ of a c.c. of blood being required for a single determination.

The principle of the method is the same as that used by Folin and Wu.[4] The advantage of using minute quantities of blood, obtained without venipuncture, is obvious, especially in work on infants and small animals. However, the accuracy of the microtechnic has been questioned in some quarters. I have, therefore, undertaken an intensive study of this method in comparison with the one originally described by Folin and Wu. This paper gives the results of this study.

PROCEDURE

One c.c. of distilled water is put into a small tube graduated at 2 c.c. The finger from which the blood is to be obtained is then washed with alcohol and ether, and pricked with a lancet. One-twentieth or $\frac{1}{10}$ c.c. of blood is drawn into a pipette, graduated at 0.05 c.c. and 0.1 c.c. Where the blood-sugar concentration is expected to be high only $\frac{1}{20}$ c.c. of blood is required. The blood is transferred from the pipette into the tube containing the distilled water. Great care must be taken in measuring the blood. After transferring the blood into the tube, the pipette must be well washed by drawing the water up into the tube several times. The tube is rolled between the palms of the hands in order to produce complete hemolysis. One-tenth c.c. of 10 per cent sodium tungstate is added, followed, after mixing, by 0.1 c.c. of $\frac{2}{3}$ N sulphuric acid to precipitate the proteins. The tube is again rolled until the mixture turns dark brown. The volume is then made up to the 2 c.c. mark and the tube again rolled. The tube is allowed to stand five minutes, then centrifuged about five minutes, and the supernatant fluid aspirated and transferred to a 10 c.c. graduated cylinder. Care must be taken not to disturb the protein precipitate. Using a pipette graduated in $\frac{1}{10}$ c.c. an aliquot (1.5 c.c. was used here) of the supernatant fluid is meas-

*From the Harry Caplin Pediatric Research Laboratory and the Pediatric Department of the Jewish Hospital of Brooklyn, N. Y.

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TABLE I

DETERMINATION OF SUGAR IN SOLUTIONS CONTAINING KNOWN AMOUNTS OF GLUCOSE*

AMT. OF SUGAR ACTUALLY PRESENT IN 100 C.C. SOL.	50 MG.		100 MG.		150 MG.		200 MG.		250 MG.		300 MG.		350 MG.	
METHOD	K–G†	F–W‡	K–G	F–W	K–G	F–W	K–G	F–W	K–G	F–W	K–G	F–W	K–G	F–W
Amt. of sugar recovered	49.8	49.3	102.7	99.0	149.3	149.4	200.3	202.6	249.1	251.5	299.0	298.5	350.5	353.8
	51.0	49.0	100.7	99.0	155.0	150.9	200.3	200.9	251.4	254.0	302.9	303.0	353.0	350.9
	48.7	50.0	100.0	95.8	151.5	151.2	200.5	195.4	249.7	253.1	301.9	296.3	350.6	347.8
	48.4	46.1	100.0	98.1	150.3	149.8	203.0	197.0	248.5	253.1	299.4	298.5	352.0	347.8
	49.0	52.4	102.8	101.0	152.3	151.5	205.0	203.2	250.9	248.4	300.9	303.0	350.5	353.8
	49.7	50.9	99.9	100.5	148.9	149.3	201.3	202.0	249.0	253.1	299.8	298.5	350.0	350.9
	50.7		104.1		152.4		200.4		250.8		303.0		349.7	
	49.3		99.4		148.9		202.4		251.8		300.6		351.8	
	50.3										300.6			
	50.7										298.0			
Av. amt. recovered	49.9	49.7	101.1	98.9	150.1	150.3	201.6	200.1	250.1	252.2	300.6	299.6	350.9	350.8
Mean error in mg. per 100 c.c.	0.1	1.5	1.4	1.3	1.8	0.2	1.7	2.7	1.1	2.7	1.4	2.4	2.2	2.3
Mean error in per cent	1.2%	3.0%	1.4%	1.3%	1.2%	0.1%	0.8%	1.3%	0.4%	1.1%	0.5%	0.8%	0.6%	0.7%
Standard deviation****	0.9	1.9	2.0	2.0	2.3	0.9	2.3	2.9	1.1	2.9	1.6	2.5	1.5	2.6

*These solutions were made up by Dr. Murray J. Shear. The author was given the values of the concentrations of these known solutions after the analyses were completed.

†K-G=Kramer-Gittleman.

‡F-W=Folin and Wu.

****In calculating the standard deviation, the amount of glucose actually present in 100 c.c. solution was used as the mean.

TABLE II

SUGAR CONCENTRATION IN BLOOD OF PATIENTS

KRAMER-GITTLEMAN		FOLIN-WU		
MG. PER 100 C.C. OF BLOOD	DIFFERENCE IN % BETWEEN CHECK DETERMINATIONS	MG. PER 100 C.C. OF BLOOD	DIFFERENCE IN % BETWEEN CHECK DETERMINATIONS	DIFFERENCE IN % BETWEEN THE MEANS OF THE TWO METHODS
A. NONDIABETIC				
85.7		85.8		
102.5		104.4		
103.7	1.2%	105.6	1.1%	1.8%
104.1		108.0		
103.9	0.2%	106.4	1.5%	3.0%
104.7		103.0		
102.9	1.7%	101.4	1.6%	1.5%
81.5		84.4		
82.9	1.7%	84.4	0.0%	2.6%
99.9		99.8		
98.7	1.2%	96.9	2.9%	1.0%
91.7		94.5		
92.4	0.8%	93.7	0.9%	2.2%
87.1		86.0		
85.6	1.7%	85.2	0.9%	0.8%
77.8		79.8		
78.0	0.3%	78.4	1.7%	1.5%
76.9		77.3		
78.1	1.5%	79.2	2.0%	1.6%
102.0		100.0		
103.0	1.0%	101.0	1.0%	1.9%
	Av. 1.1%		Av. 1.3%	Av. 1.8%
B. DIABETIC				
400.0		400.0		
396.7	0.8%	400.0	0.0%	0.4%
253.8		256.4		
256.4	1.0%	259.7	1.2%	0.4%
280.1		277.8		
278.5	0.6%	276.7	0.4%	0.7%
209.7		207.0		
208.9	0.4%	206.1	0.4%	1.1%
216.5		216.4		
216.0	0.2%	216.4	0.0%	0.2%
	Av. 0.6%		Av. 0.4%	Av. 0.6%

ured into a modified Folin-Wu sugar tube graduated at 4 c.c. and 6 c.c. Two standards are prepared using 1 c.c. and 0.5 c.c. respectively of a solution containing 0.1 mg. of sugar. Two c.c. of the alkaline copper solution are added to each tube and the volume is made up to 4 c.c. The tubes are heated in a boiling water-bath for six minutes, transferred to a cold water-bath and allowed to stand three minutes. If the tubes are heated longer than six minutes, reoxidation takes place, and low results are obtained. Two c.c. of the phosphomolybdate solution are added to each tube and the volume is made up to 6 c.c. After letting the tubes stand from five to ten minutes to permit the escape of CO_2 gas, the solutions are ready to be read in

TABLE III

SUGAR CONCENTRATION IN BLOOD OF DOGS
IN MG. PER 100 C.C. OF BLOOD*

DOG 52		DOG 53		DOG 54		DOG 57		DOG 61	
K–G	F–W	K–G	F–W	K–G	F–W	K–G	F–W	K–G	F–W
102.6	109.0	81.2	78.4	87.1	88.1	87.3	86.0	94.9	94.0
106.4	109.0	78.8	78.4	90.1	87.4	85.4	87.5	93.5	94.5
128.7	129.0	124.2	129.0	100.0	98.4	81.3	83.4	82.4	81.3
126.5	129.0	123.8	128.6	101.2	98.4	82.98	83.4	83.3	82.0
		110.3	112.3					87.2	86.0
191.3†	192.3	107.1	113.0	103.0	101.6	87.1	87.0	88.2	87.1
192.9	194.1			103.0	105.0	87.1	86.0	85.0	86.0
161.3	163.9	112.8	115.6	102.4	104.2	85.7	81.05	86.2	86.0
164.7	165.2	115.2	114.2	104.0	105.2	83.9	82.0	73.0	71.6
144.9	147.0	150.9†	155.0	104.0	105.0	81.8	83.4	75.7	73.2
146.3	147.0	150.9	154.2	103.0	105.0	80.3	82.6	71.1	72.5
137.6	138.2	153.0	158.0	103.0	105.7	74.1	73.2	72.1	72.5
135.2	137.9	150.9	154.2	106.0	104.2	71.9	74.0	39.1‡	38.5
127.1	129.0	148.7	150.0	74.0	74.5	22.7‡	20.0	35.0	33.6
125.3	129.0	146.7	150.0	33.3‡	34.0	24.3	20.7		
101.3	99.5	108.1	107.0	31.0	32.8				
		119.6	121.2						
125.0	122.6	124.3	121.5						
125.0	124.2	122.6	121.9						
		119.6	121.2						

*These blood-sugar determinations were made in connection with the study of the effect of ligation of the hepatic artery on carbohydrate metabolism.[5]

†Blood-sugar determinations after injection of adrenalin.

‡Blood-sugar determinations following hypoglycemic shock.

the colorimeter. The standard is set at 15 and read against itself. If the colorimeter is properly adjusted, the two standards should give the same reading. One standard is then replaced by an unknown and several readings taken. While the solutions are being compared in the colorimeter, care should be taken that no gas bubbles collect about the prisms.

Calculations—

$$\frac{\text{Reading of standard} \times \text{mg. of glucose in standard} \times 100}{\text{Reading of unknown} \times \text{amount of blood present in the aliquot}} = \text{mg. of sugar per 100 c.c. of blood}$$

e.g.

When 0.1 c.c. of blood is used, 1.5 c.c. of supernatant fluid represents 0.075 c.c. of blood. If this gives a reading of 20 against a 0.1 mg. standard set at 15, then the calculation is as follows:

$$\frac{15 \times 0.1 \times 100}{20 \times 0.075} = 100.0 \text{ mg. of sugar per 100 c.c. of blood.}$$

or to simplify the calculation, divide 2000 by the reading:

$$\frac{2000}{\text{Reading}} = \text{mg. sugar in 100 c.c. of blood}$$

$$\frac{2000}{20} = 100 \text{ mg. of sugar per 100 c.c. of blood.}$$

RESULTS

Table I gives the results of a series of comparative determinations by the original Folin-Wu method and by the Kramer-Gittleman modification on solutions of known concentrations ranging from 50 mg. to 350 mg. per 100 c.c.

The average amount of glucose recovered in each solution is shown. The mean error has been determined in terms of milligrams per 100 c.c. and per cent. Using the amount of glucose known to be present as the mean, the standard deviation from this quantity has been calculated.

Table II, first and third columns, gives the results of comparative determinations by the Folin-Wu method and by the Kramer-Gittleman method on the blood of a series of nondiabetic and diabetic patients. The difference in per cent between check determinations with each method is given in the second and fourth columns respectively. The difference in per cent between the average values obtained with these two methods is given in the last column.

Table III shows a series of comparative determinations made on the blood of dogs. These dogs were used in a study of the relation of the blood-sugar level to the convulsive seizure following ligation of the hepatic artery.

CONCLUSIONS

1. The average reproducibility of the Kramer-Gittleman method is about 1.0 per cent; that of the Folin-Wu is also about 1.0 per cent.

2. The values obtained by the Kramer-Gittleman technic agree with those obtained by the Folin-Wu technic to within 2.0 per cent. This is only slightly greater than the degree of reproducibility of either method.

3. These findings show that the microtechnic of Kramer and Gittleman for the quantitative determination of substances in blood that reduce alkaline cupric hydroxide solutions gives results that compare well with those obtained with the original method of Folin and Wu.

The author is indebted to Dr. Benjamin Kramer for his advice and encouragement in this investigation.

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NORMAL BLOOD COUNTS IN PIGEONS*

By Floyd De Eds, Ph.D., San Francisco, Calif.

DURING the course of studies on the anaphylactoid and anaphylactic shock reactions in pigeons[1] in this laboratory a large amount of data on normal blood counts has been accumulated. In view of the limited information on this subject available in the literature, it seemed worth while to place this data on record.

I am familiar with only two reports on pigeon blood in the literature. Klieneberger and Carl[2] made a study of the blood morphology of six pigeons. They reported the following counts: erythrocytes from 3,780,000 to 4,535,000; leucocytes from 10,430 to 31,430; and thrombocytes (platelets) from 9,070 to 63,490 per cu. mm. The differential leucocyte count also showed great variability. A similar variability in cell counts has been reported by Arloing and Dufourt,[3] but they do not state the number of pigeons used.

The blood counts reported in this paper were made on normal pigeons using blood from two regions, heart blood, i.e., blood from the heart by chest puncture, which represented the main channel of the circulation, and blood from a superficial vein of the leg, which represented a peripheral channel. For differential staining Hastings'[4] modification of the Nocht stain

Table I

Normal Blood Counts in Pigeons

Type of cell	Heart		Leg vein	
	Range	Median	Range	Median
Small lymphocytes	5 to 53%	36% (23)*	2 to 73%	30% (33)*
Large lymphocytes	9 to 67%	21% (23)	3 to 61%	27% (33)
Pseudo eosinophilic polymorphonuclears	0 to 25%	3% (23)	0 to 31%	1% (33)
Eosinophilic polymorphonuclears	2 to 75%	26% (23)	14 to 55%	27% (33)
Eosinophilic myelocytes	0 to 21%	0% (23)	0 to 4%	0% (33)
Basophilic myelocytes	0 to 3%	0% (23)	0 to 2%	0% (33)
Basophilic polymorphonuclears	0 to 2%	0% (23)	0 to 7%	0% (33)
Leucocytes	7,600 to 16,600	11,800 (6)	3,600 to 8,600	5,200 (6)
Erythrocytes	2,625,000 to 4,325,000	3,300,000 (25)	2,275,000 to 4,475,000	3,350,000 (26)
Thrombocytes	8,000 to 89,000	24,000 (32)	8,000 to 84,000	38,000 (45)

*Number of pigeons used in parentheses.

*From the Department of Pharmacology, Stanford University School of Medicine, San Francisco, Calif.

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was employed. For counting, the diluting fluid of Rees and Ecker[5] was used for erythrocytes, thrombocytes and leucocytes; the erythrocytes remained unstained and all other cells were stained blue. The results obtained are presented in the accompanying table.

The results confirm the great variability in blood counts of pigeons previously reported by others. The differential counts on leucocytes were originally made with the view of correlating certain changes in these cells with the anaphylactoid and anaphylactic shock reactions just as had been done with the erythrocytes and thrombocytes in a previous study,[1] but the counts were too variable to permit the drawing of definite conclusions. This was true of the leucocyte counts in the experiments individually and collectively. In the previous study,[1] the counts of thrombocytes and erythrocytes relative to each other were more uniform and permitted definite conclusions. It is obvious that caution should be exercised in drawing conclusions from blood counts in pigeons.

CONCLUSIONS

Normal blood counts on a large number of pigeons are reported, and the great variability previously reported confirmed.

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A STUDY OF THE PIGMENT IN ADDISON'S DISEASE*

By Carl L. Spohr, M.D., and Robert A. Moore, A.B., Columbus, Ohio

MELANIN occurs normally as the coloring matter of the hair, of the choroid of the eye, of the skin and in the pigment matter of many lower animals.

Its function is evidently that of protection from light rays. Melanin seems to be produced through the metabolic activity of specialized cells and old theories concerning its origin from hemoglobin have been abandoned.

Because of some unusual activity melanin accumulation may occur somewhere within the body in excess. While the entire skin of a negro is said to contain only about one gram of melanin, excessive quantities may be deposited in the lymph nodes, skin, etc.

Addison's disease is usually associated with a deposition of a brownish pigment in the skin and occasionally in other organs. Von Furth and his coworkers have shown a definite relationship of tyrosine and melanin and other investigators have, at least partially, shown a relationship of tyrosine

*Read before the Fifth Annual Convention of the American Society of Clinical Pathologists at Dallas, Texas, April 15, 16 and 17, 1926.
From the Department of Pathology, the Ohio State University, Columbus, Ohio.

and adrenalin. On this basis it has been assumed that the pigment of use in Addison's disease is a melanin and is due to some dysfunction in the metabolism of the aromatic radicles. As yet no definite chemical evidence has been brought forward to prove the melanotic character of this pigment.

During the spring of 1925 it was our good fortune to perform a necropsy (on the pathologic service of the Ohio State University) on a typical case of Addison's disease with pigmentation of the skin and active tubercular caseation of the right adrenal and old tubercular fibrosis of the left adrenal. The point of greatest interest was that the entire lymphatic system of the abdomen and chest was heavily pigmented black. The case report and histologic findings will be reported elsewhere, suffice it to say at this time that it was early realized that the pigment was not an anthracosis. Microchemical reactions showed that the pigment was iron free and was bleached by sunlight and hydrogen peroxide. In accord with this idea a chemical investigation of the pigment was thought to be pertinent.

Isolation of the Pigment.—The method of Gortner[2] using 0.2 per cent NaOH was followed very closely. In brief this method is as follows: The lymph glands were finely ground in a mortar and placed in a flask with an excess of 0.2 per cent NaOH. The whole was boiled under a reflux condenser for three hours. The supernatant fluid was poured off and the process of extraction repeated. The two extracts were combined, filtered through a Buchner filter and concentrated HCl added until a coarse flocculent precipitate appeared. The whole was centrifuged and the supernatant poured off. The dark mass remaining was dissolved in N/20 HCl and again centrifuged and the melanin solution poured off. Concentrated HCl was added until a flocculent precipitate appeared. (Gortner recommends adding up to 1 per cent but we find that this is not sufficient acid to cause the precipitation of the melanin. Titration results on the fluid show the concentration to be about 2.5 per cent.) The precipitate is centrifuged and the acid poured off. The precipitate is dissolved in an excess of 50 per cent acetic acid and centrifuged. The dark supernatant solution is placed in a celloidin dialyzing sac and dialyzed until the melanin is precipitated and the dialyzate is free of chlorides. Centrifuge the suspension and collect the precipitate in a porcelain dish. Dry on a water-bath. The dry black powder is extracted in a Soxhlet with carbon bisulphide, alcohol and finally ether. This dry powder was used in the analytical results reported below.

Amount of Pigment.—Unfortunately the lymph glands were not weighed at the start and only a very general idea of the amount of pigment can be secured when it is stated that the entire lymphatic apparatus of the thorax and abdomen yielded about 0.7 gm. of pigment.

Ash Content.—The pigment was placed in a platinum crucible and heated until all organic matter was oxidized.

0.1236 gm. of pigment yielded 0.0011 gm. of ash, or 0.93 %.

Sulphur.—Liebig's alkali method was used as outlined by Sherman.[3] An alcohol lamp and nickel apparatus was used for the fusion. The barium sulphate was washed, ignited and weighed as outlined by Foulk.[4]

0.3054 gm. of pigment yielded 0.0683 gm. of barium sulphate. Calculated on an ash free basis this is 3.10 per cent S. (0.0032 gm. of barium sulphate was secured in a blank.)

Nitrogen.—This constituent was determined by the general Kjeldahl method outlined by Foulk.[4]

0.0174 gm. of pigment gave off the ammonia equivalent of 5.48 c.c. of N/50 HCl. Calculated on an ash free basis this is 11.24 per cent considering that 1 c.c. of 0.02 N ammonia equal 0.000341 gm. ammonia.

Carbon and Hydrogen.—These two elements were determined in a combustion train according to the methods outlined by Gatterman[5] for substances containing nitrogen and sulphur. The combustion was carried out with lead chromate and a reduced copper spiral.

0.2150 gm. pigment yielded 0.1064 gm. water and 0.4287 gm. carbon dioxide. Calculated on an ash free basis this is

C 54.8 per cent
H 5.56 per cent

SUMMARY

From these results we can formulate:

C	54.8	per cent
H	5.56	per cent
S	3.1	per cent
N	11.24	per cent
O	25.4	per cent (by difference)

Calculated on this basis the simplest possible formula is

$$C_{50}H_{62}N_9SO_{20}$$

DISCUSSION

As these results appear to us, the exact amounts of each substance are only of value to a slight extent for as has been pointed out by Gortner[2] it is impossible to prepare two samples of melanin from the same source and secure the same figures.

The significance of these results lies mostly in the sulphur determinations. Granting that the Liebig alkali method gives consistently high results even on known chemical compounds, the value of 3.10 per cent after a blank determination has been subtracted points most clearly to the fact that this pigment is not related to any known derivative of hemoglobin formed either by changes in the animal body or by chemical processes outside the body, since none of these derivatives have ever shown a higher percentage than 0.5 per cent of S.

In the isolation of the pigment it was noted that the two types of melanin mentioned by Gortner[2] were present, that is, one soluble in weak acid and one insoluble in weak acid. The amount of the latter was so small that any analytic work was impossible.

The comparison of the results of the chemical analysis of this pigment with that of melanins derived from other sources by different authors is not of any value. As has been pointed out by Gortner[2] the actual chemical percentages of the elements vary considerably with the method of extraction. But

since we have followed the method used by Gortner in detail, it follows that a comparison with the results of his analysis using 0.2 per cent NaOH to extract the pigment from black wool is justifiable.

	C	H	N	S
Gortner	52.60	7.28	13.52	1.33
This paper	54.8	5.56	11.24	3.10

Although the differences are well beyond the limits of experimental error and beyond the errors secured by us in the analysis of known chemical compounds with the same apparatus and chemicals, we believe that, considering the possibility of slight variations in the technic of purifying the pigment and in the possibility of several different melanins existing, the figures are comparable, with the exception of the sulphur. Here some of the differences may be accounted for on the basis of the Liebig method used by us, but the remainder must remain unaccounted for until further observations can be made in other cases of Addison's disease.

SUMMARY

1. The ultimate chemical analysis of a pigment derived from the lymph glands in a case of Addison's disease is reported.

2. The evidence seems to point that this pigment is a melanin and the absence of any other explainable cause of the melanosis than the Addison's disease would lead one to believe that there was more than a casual relationship between the two.

3. We believe that the pigment laid down in the tissues is a melanin, and that it bears a direct relationship to the condition responsible for the pathologic complex of Addison's disease.

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DISCUSSION

Dr. Wm. G. Exton.—It really represents a tremendous amount of work. I am not quite clear, Dr. Spohr, about the final figures, isn't that a little out of the way?

Dr. Carl Spohr.—Our figures were higher than those given by Gortner.

Dr. Wm. G. Exton (continuing).—I wonder if that is close enough. We got consistent results showing that the method by Gortner was a good one. What I wanted to do was to call attention to the vast amount of work in this.

EXPERIMENTAL BACILLUS PYOCYANEUS KERATITIS*

By Edna Jackson, M.A., and F. W. Hartman, M.D., Detroit, Mich.

THE pathogenicity of B. pyocyaneus for laboratory animals is well recognized but it is generally regarded as of low virulence in man. There are recorded, however, a few isolated eye infections of most serious character. As in the reports of Mauersberg, Kritzky and Lamb and Calhoun, several of the observers have reproduced the lesion in the eyes of rabbits.

The virulent strain of B. pyocyaneus used in this study was isolated from seven of a group of eighteen industrial cases. The detailed report of these cases and the review of those appearing in the literature will appear elsewhere.

Of the eleven eyes from which pyocyaneus was not isolated, staphylococcus aureus was isolated in two, staphylococcus albus in two, two cultures were negative and five were not cultured for various reasons. After two successive cultures showed pyocyaneus the infected swabs were used to injure the cornea of rabbits' eyes in an attempt to reproduce the disease. This attempt failed and further experiments included six groups, all under cocaine anesthesia, as follows:

1. *Injection of the culture between the layers of the cornea.*—As the rubbing of the cornea with an infected swab gave no results, and as the clinical cases gave history of injury to the eye before infection, infection was attempted by an inoculation which would injure the cornea and place some of the organisms between the corneal layers. The growth from a twenty-four hour agar slant culture of B. pyocyaneus was washed off with 5 c.c. of sterile saline. By means of a fine hypodermic needle, inoculations of this suspension were made into the cornea of both eyes of two rabbits and of one guinea pig. A lesion about 2 mm. in diameter was thus produced. At the end of twenty-four hours there had developed from each inoculation a marked conjunctivitis, and at the site of each inoculation an ulcer had developed which already showed marked digestion. Pus was present in quantities, making the opening of the eyelids difficult. In forty-eight hours the ulcers had spread over a much larger area, the affected part being opaque, showing digestion and sloughing of the tissue. At the end of four days the ulcers involved the entire cornea in the rabbits, and in the guinea pig so much sloughing had occurred that the anterior chamber of the eye had evacuated itself. Cultures taken from the ulcers at the end of three days showed B. pyocyaneus in pure culture.

Control sterile needle punctures into the cornea caused a slight conjunctivitis which quickly subsided, and at the end of twenty-four hours the point of puncture was scarcely visible.

*Read before the Fifth Annual Convention of the American Society of Clinical Pathologists at Dallas, Texas, April 15, 16 and 17, 1926.

From the Laboratories of the Henry Ford Hospital, Detroit, Mich.

TABLE I

EX. I. INJECTION OF CULTURE OF B. PYOCYANEUS BETWEEN LAYERS OF CORNEA

	24 HOURS	48 HOURS	4 DAYS
Rabbit No. 1	Large spreading ulcers. Conjunctivitis. Pus.	Large deep spreading ulcers. Conjunctivitis. Profuse pus formation. Sloughing of cornea.	Deep undermining ulcers, covering entire visible portion of eye. Conjunctivitis. Pus.
Rabbit No. 2	Large spreading ulcers. Conjunctivitis, marked. Profuse pus formation.	Large ulcers, spreading. Conjunctivitis. Profuse pus formation. Affected portion opaque.	Deep ulcers covering entire visible portion of eye. Conjunctivitis. Pus. Whole cornea opaque.
Guinea Pig No. 3	Ulcers rapidly spreading. Marked conjunctivitis. Profuse pus formation.	Ulcers, spreading. Conjunctivitis. Profuse pus formation. Digestion marked. Sloughing of cornea.	Ulcers covering entire visible portion of eye. Marked digestion and sloughing. Large amount of pus and exudate.
Rabbit No. 3-a Control-sterile needle puncture	Very slight conjunctivitis.	Negative.	Negative.

2. *Instillation of pyocyaneus cultures into the conjunctival sac.*—Another attempt was made to produce infection by instilling a saline suspension of a twenty-four-hour culture of a virulent strain into the conjunctival sac of the eyes of two rabbits. At no time could any effect from these instillations be observed.

3. *Scratching the cornea and instilling the culture into the conjunctival sac.*—In this experiment six rabbits were used; in three, corneal layers of eyes were scratched with a needle and in three the corneal layers were scratched by means of fine iron filings. One of each set was kept as a control. Cultures of B. pyocyaneus were instilled into the conjunctival sac of the others. Where iron filings were used the results were negative. Where the cornea was scratched with a needle a slight conjunctivitis developed which disappeared in two or three days. No ulcers formed, and the injuries were scarcely visible in twenty-four hours.

4. *Injection of broth filtrate into the corneal layers.*—Questioning the rôle that the proteolytic ferments present in pyocyaneus filtrates might play in the production of these digesting ulcers, we injected sterile broth filtrates from six-day-old cultures into the cornea of both eyes of two rabbits. The inoculations were made by means of a hypodermic needle as in the first experiment. From these inoculations there resulted a marked conjunctivitis, also slight pus formation. No ulcers formed and there was no evidence of digestion. The conjunctivitis subsided, and in four days the eyes appeared normal except for the minute lesions at the site of inoculation.

5. *Inoculations of Staphylococcus aureus and Staphylococcus aureus plus sterile broth filtrates of B. pyocyaneus.*—The staphylococcus culture used in the experiment was one which had been isolated from one of the severe clinical cases. Saline suspensions were made from a twenty-four-hour agar slant culture, and needle inoculations were made into the corneal layers of both

eyes of two rabbits. In twenty-four hours there was a marked conjunctivitis, a moderate amount of exudate present and small staphylomas at the points of inoculation. These lesions showed no digestion and there was no extension of the lesions. In four days the eyes of one of the rabbits were apparently normal, while one of the eyes of the other rabbit showed a small staphyloma. There was no evidence of digestion or of a tendency to spread.

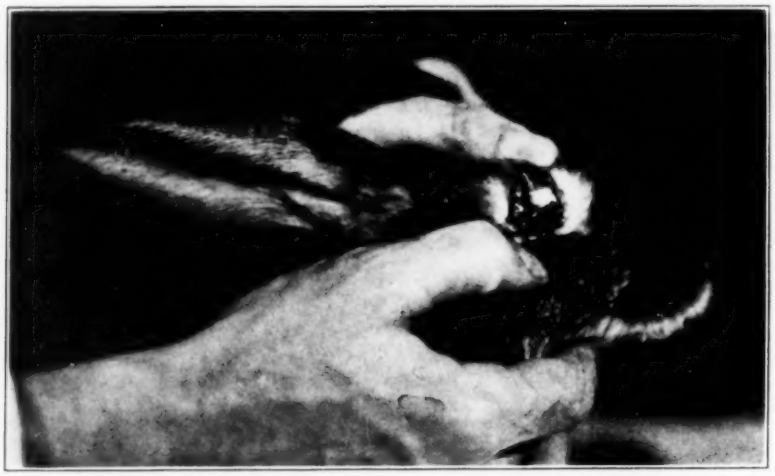

Fig. 1.—Staphyloma at the site of inoculation with conjunctivitis.

Fig. 2.—Shows complete undermining and opacity of cornea.

It seemed of interest to determine whether the staphylococcus could, in the presence of the pyocyaneus filtrate, produce lesions similar to those produced by the inoculation of B. pyocyaneus. The eyes of two rabbits were injected as above, using a mixture of equal parts of the saline suspension, of staphylococcus and of sterile filtrate from pyocyaneus broth cultures.

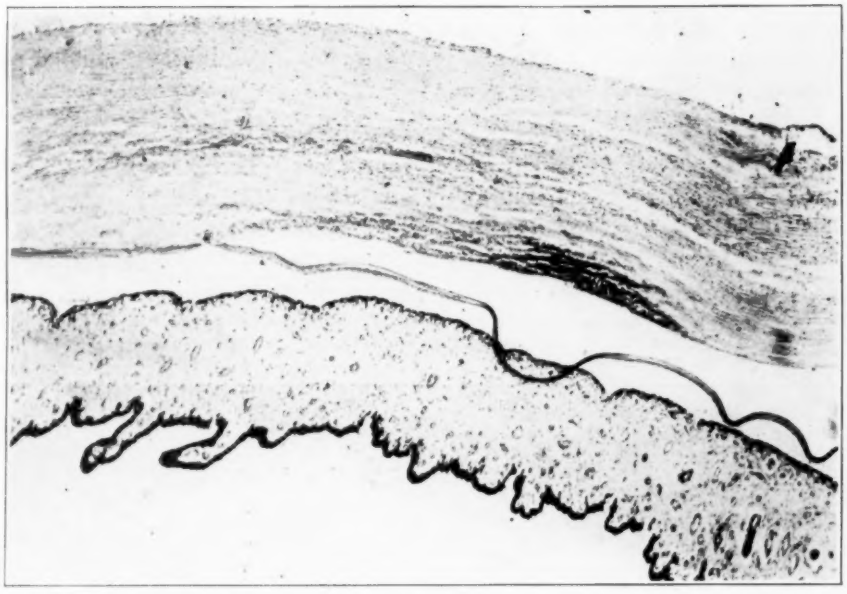

Fig. 3.—Microphotograph with ⅔ objective showing moderate diffuse infiltration of the corneal layers with separation of the same.

Fig. 4.—Microphotograph with ⅙ objective shows partial separation of Bowman's capsule and intense infiltration of the layers of the cornea. The adjacent eyelid is also infiltrated with round, wandering cells and polymorphonuclear leucocytes.

In these cases the resulting conjunctivitis was more marked than when staphylococci were injected alone; there was also more exudate present and evidence of slight digestion. The lesions, however, did not spread and gradually the conjunctivitis subsided so that in a few days the only evidences of the infection were small lesions at the points of inoculation.

6. *Injection of old laboratory strains of pyocyaneus.*—Two old laboratory strains of pyocyaneus were obtained through the courtesy of Parke-Davis & Company. Saline suspensions of twenty-four-hour old cultures were injected into the corneal layers of two rabbits. The lesions following injection of one strain showed considerable digestion and marked conjunctivitis, but no typical ulcers developed and there was no extension of the lesions. The conjunctivitis was marked for several days; then it gradually subsided. The lesions

Fig. 5.—Shows microphotograph with ⅔ objective, intense infiltration of the cornea and exudate in the anterior chamber of the eye.

showed no ulceration or extension. The reaction from inoculation of the second strain was less marked. There was a slight conjunctivitis and small localized lesions which showed no digestion and no spreading. These laboratory strains possess only a low virulence for guinea pigs; a half c.c. of saline suspension injected intraperitoneally into 250 gm. guinea pigs did not kill. The animals were somewhat affected for two days but all recovered. The injection of $\frac{1}{20}$ c.c. of our freshly isolated strains always killed within two days. Attempts to raise the virulence of the laboratory strains by means of passage from one guinea pig to another were not successful.

Whether or not there is any relation between virulence and pigment fermentation, it is interesting to note that in the old laboratory strains there was no chloroform-soluble bluish-green pigment present. The agar slant cul-

TABLE II

EX. VII. IMMUNIZED RABBITS INJECTED WITH CULTURES OF B. PYOCYANEUS

	24 HOURS	48 HOURS	4 DAYS	PROTECTION
Rabbit No. 19. (Immunized with B. pyocyaneus cultures.)	Marked conjunctivitis. Small lesions. Pus present. No digestion. No extension.	Marked conjunctivitis. Small localized lesions. Small amount of pus.	Little conjuctivitis. Very slight lesion at points of inoculation. No pus.	Marked
Rabbit No. 20. (Immunized with B. pyocyaneus cultures.)	Marked conjunctivitis. Small amount of pus. No digestion.	Conjunctivitis subsiding. Lesions small, not extending.	Little conjunctivitis. Small localized lesions, at point of inoculation.	Marked
Rabbit No. 21. (Immunized with B. pyocyaneus filtrate.)	Marked conjunctivitis. Small lesions. Pus present. No digestion, observed.	Marked conjunctivitis. Lesions somewhat extended. Large amount of pus.	Conjunctivitis less. Lesions show no further extension. Small amount of pus. No sloughing.	Moderate
Rabbit No. 22. (Immunized with B. pyocyaneus filtrate.)	Marked conjunctivitis. Lesions small. Large amount of pus.	Marked conjunctivitis. Lesions somewhat extended. Large amount of pus. No deep ulcerations.	Conjunctivitis subsiding. Lesions localized, no further extension—larger than in Rabbits 19 and 20.	Moderate
Rabbit No. 23. Control	Marked conjunctivitis. Definitely spreading ulcers. Large amount of pus.	Marked conjunctivitis. Marked digestion. Large amount of pus. Ulcers extending.	Marked conjunctivitis. Visible portion of eye opaque. Large amount of pus. Sloughing.	

TABLE III

EX. VIII. RABBITS INJECTED WITH CULTURES OF B. PYOCYANEUS AND RECEIVING SERUM FROM IMMUNIZED RABBIT

	ANTI-CULTURE SERUM	24 HOURS	48 HOURS	4 DAYS
Rabbit No. 24	25 c.c. i.v. 18 c.c. at end of 24 hours	Marked conjunctivitis. Much pus. Lesions slightly enlarged.	Marked conjunctivitis. Small amount of pus. No extension.	Slight conjunctivitis. No pus. Small lesions at point of inoculation.
Rabbit No. 25	25 c.c. i.v. 15 c.c. 24 hours	Marked conjunctivitis. Large amount of pus.	Marked conjunctivitis. Pus. Some extension of lesion in left eye.	Slight conjuctivitis. Pus. No further extension of lesion. No erosion.
Rabbit No. 26	Control	Marked conjunctivitis. Large amount of pus. Some digestion.	Marked conjunctivitis. Large amount of pus. Ulcers spreading. Digestion marked.	Conjunctivitis. Large amount of pus. Visible portion of eye opaque. Sloughing.

tures appeared somewhat fluorescent but they lacked the typical bluish-green pigment of the freshly isolated cultures. Agar slants of these cultures are distinctly bluish-green and the pigment is easily extracted by chloroform.

7. *Since clinically the only effective treatment tried was actual cautery, it seemed worth while to investigate the possibility of active and passive immunity.*—For this purpose two groups of rabbits were taken. One group was injected with increasing doses of a killed culture of B. pyocyaneus, the other with sterile filtrates of six-day broth cultures of B. pyocyaneus. The killed

cultures used were obtained by washing the growth from a twenty-four-hour agar culture, then washing this once with sterile saline. The sediment was taken up in sterile saline and heated at 60° for forty-five minutes. Because of the difficulty often encountered in giving injections of pyocyaneus, small doses were given, beginning with doses of 0.2 c.c. of the culture and filtrate, slowly increasing the amount until 0.75 c.c. was given in the eighth injection. After the eighth injection samples of blood were taken from one of each group to test the titer of the serum. The serum of the rabbit which had received the culture injections completely agglutinated the pyocyaneus organisms in a dilution of 1-640, incompletely in a dilution of 1-1280 (two hours in the water-bath and overnight at room temperature). The serum from the rabbit which had received the injections of filtrate was tested against a sterile

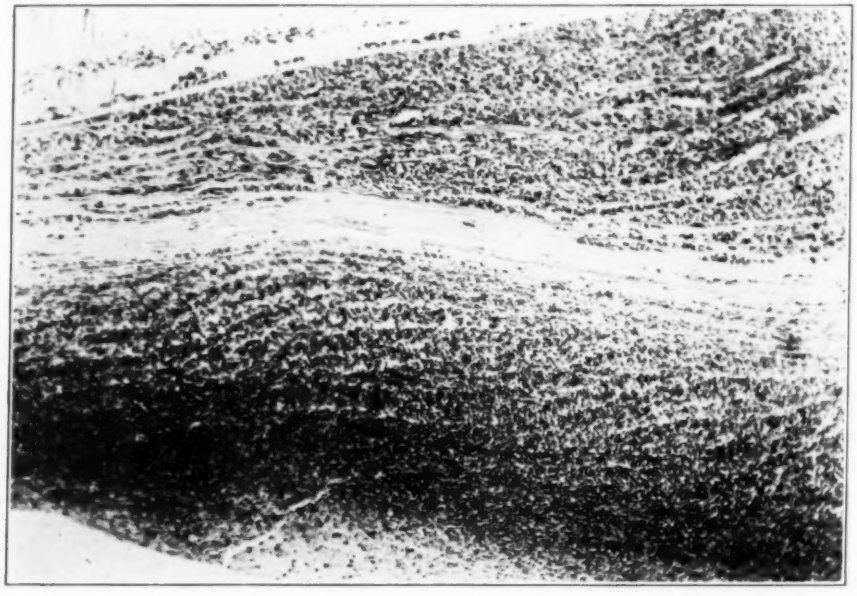

Fig. 6.—Microphotograph with ⅙ objective, same area showing character of infiltration of the cornea.

broth pyocyaneus filtrate for the presence of precipitins and against saline suspensions of pyocyaneus for the presence of agglutinins. No precipitin reaction was obtained but the serum agglutinated the pyocyaneus culture in a dilution of 1-320. These rabbits were bled and their serum used in an experiment described later.

Two rabbits which had received the injection of killed cultures and two which had received the filtrate injections were inoculated with a twenty-four-hour culture of pyocyaneus, a control rabbit being injected at the same time. These inoculations were made by hypodermic needle into layers of cornea as before.

The control rabbit developed the typical spreading lesions which have been described above. The purulent conjunctivitis persisted at end of two weeks. The ulcers extended gradually, covering the cornea. The rabbits

immunized by killed cultures developed, after the inoculation of the eyes, a marked conjunctivitis; lesions formed at site of inoculation but these showed little tendency to spread. There was no ulceration. At the end of four days the conjunctivitis had about subsided; there remained only very small lesions at the site of inoculation. The rabbits which had been inoculated with the sterile filtrate from broth cultures of pyocyaneus developed more destructive lesions than those which had been immunized with cultures.

From this small number of animals it would seem that a better immunity was obtained by injection of cultures than by injection of filtrates.

The two rabbits whose serum had been tested for agglutinizing titer were bled to death, the serum being used to passively immunize other rabbits.

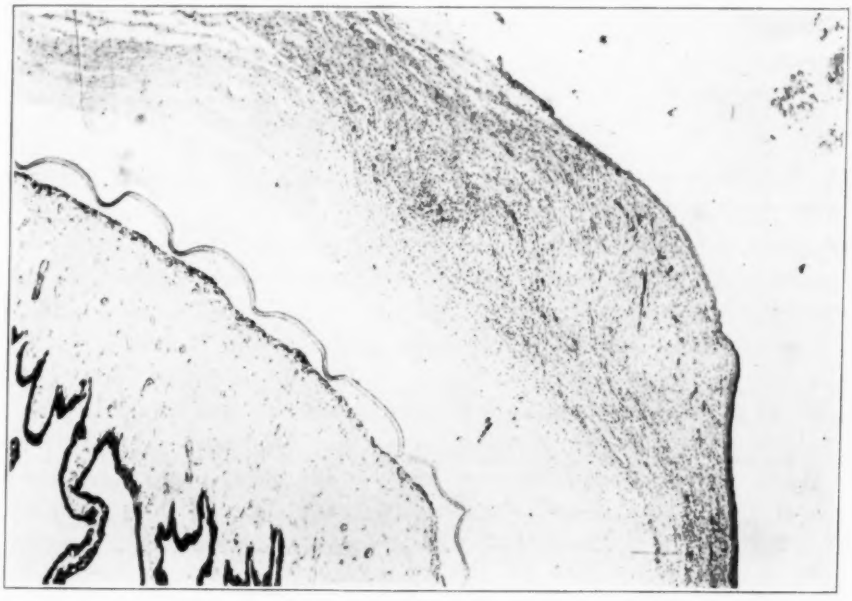

Fig. 7.—Shows microphotograph with $\frac{2}{3}$ objective with area of dense scarring in the cornea.

Three rabbits were injected with a twenty-four-hour culture of B. pyocyaneus by needle inoculation into the cornea. One of the rabbits inoculated was given 25 c.c. of serum from the rabbit which had been immunized by injection of killed cultures. In twenty-four hours there was little difference to be noted in the reactions in the two rabbits. (A second intravenous injection of 20 c.c. of serum was given.) In forty-eight hours the eye of the control rabbit showed marked conjunctivitis, profuse exudation, the lesions were spreading rapidly. In the rabbit which had received the immune serum the lesions were small and they showed no extension. The conjunctivitis which had been so marked was receding and there was little exudate present. At the end of four days the conjunctiva was about normal, there was no pus and the lesions at the site of inoculation were very small and definitely localized. The lesions of the control rabbit had extended, showed more digestion and there was still

a large amount of pus present. The third rabbit received 20 c.c. of immune serum from the rabbit which had received the injection of sterile broth filtrate and 18 c.c. again at the end of twenty-four hours. In this rabbit little evidence of protection was noted. The lesions which developed were of the same severity as those of the control, the spreading of lesions and digestion of tissue did not seem to be affected by the serum.

SUMMARY

1. Keratitis was experimentally produced by B. pyocyaneus, the inoculations being made into the corneal layers.

2. Attempts to produce keratitis were not successful:

(1) When instillations of B. pyocyaneus were made into the conjunctival sac.

(2) When pyocyaneus filtrates were injected.

(3) When cultures of staphylococci were injected into corneal layers.

(4) When cultures of old laboratory strains were injected into corneal layers.

3. Rabbits were effectively immunized by injection of killed cultures of B. pyocyaneus and by injection of pyocyaneus filtrates.

4. Rabbits receiving immune serum were not effectively protected except in one case. Favorable results in this case suggest that an effective immune serum could be produced.*

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*Antiserum of high titre is now available from Parke, Davis and Co., Detroit.

INTESTINAL AMEBIASIS FROM THE PATHOLOGIST'S STANDPOINT AS RELATED TO THE CLINICAL PICTURE*

By J. M. Feder, M.D., Grand Junction, Colo.

INTESTINAL amebiasis was long considered a disease almost wholly tropical, very few cases occurring within the temperate zone were recorded; however, within the past decade the number of cases manifesting themselves is steadily rising. That this disease is by no means a rarity in the temperate zone can be borne out by the figures of Kofoid and others which showed that 3 per cent of all soldiers serving in the United States were infected with E. histolytica and that 10.8 per cent of those returning from France were similarly infected. Then, recognizing that intestinal amebiasis is a cosmopolitan disease it is felt that we must be more and more on the alert to detect it, both in the patient suffering from the disease and in the carrier. In the preparation of this paper we have constantly kept the clinical pathologist in mind as the pivot upon whom the control of this condition must turn and to whom the clinician will come with his problems of differential diagnosis and later for treatment control. We have endeavored to keep constantly in view the fact that this is not a symposium on protozoology or pathology, but rather an attempt to crystallize into as short a space as possible, this as a practical subject in a manner acceptable to practical men.

We have purposely omitted all cumbersome data and have boiled the text down to proved facts. Charts and specimens have been used where it is considered that they would replace a lengthy description. Many facts and figures herein included have been drawn from the experiences of others and as the sources have been so numerous it is not possible to give due credit in each case. No one was in a better position to study this condition than the pioneer physicians in the Panama Canal Zone and their work will endure for all time as a most valuable contribution to protozoology. Those physicians found amebiasis rampant during the early days and soon found that its immediate reduction or eradication was imperative. I recently heard a local authority down there state that at that time intestinal amebiasis was one of the most widely spread protozoal diseases and that malaria was not excluded.

We will now endeavor to demonstrate that with the coming of sanitation the number of cases on the Canal Zone dropped to almost zero. It is noted that there is a great decrease in the number of cases coming to autopsy at the Ancon Board of Health Laboratory from 1905 to 1923. It will be noted that the cases have fallen from 50 cases of amebiasis to the 1000 autopsies in 1905 to 4 cases to the 1000 in 1923. Undoubtedly the coming of better

*Read before the Fifth Annual Convention of the American Society of Clinical Pathologists at Dallas, Texas, April 15, 16 and 17, 1926.

hygienic conditions was responsible for this drop on the Canal Zone. At the Santo Tomas Hospital in the Republic of Panama where our patients are drawn from the interior, the fall in rate incidence has not been so spectacular. Reliable statistics are not available for the same period in this institution but at present about 2 per cent of all cases coming to autopsy show gross lesions of amebiasis, many more microscopically.

Now that we have demonstrated that the number of cases fell in ratio to improved sanitary measures, let us consider the mode of transmission in order to more clearly appreciate the hygienic procedures required.

Man can become infected with E. histolytica in only one way, and that is to swallow the cysts of the organism, and the concensus of opinion among us is that those cysts are ingested by the accidental contamination of food or drink by minute particles of feces containing them and that while the particle of feces may be dry on the surface, its moist center protects living cysts. It is believed that the carrier problem is the biggest factor in the transmission of the disease and that contaminated water supplies play a minor rôle if any. It has been the experience in Panama that cases following flooding of wells and other known contamination of water supplies are rare and that the dysenteries following such contamination are usually bacillary in origin. In a country where amebiasis is endemic, one would naturally expect to find some epidemics following these accidents if the disease were easily transmitted by water supplies. We do at times, however, find the two dysenteries existing in the same individual following these exposures, but as an attack of bacillary dysentery frequently brings about an acute exacerbation in a case of chronic amebiasis, little import is attached to them from the standpoint of epidemiology.

The opportunity to prove the carrier theory has been given many times; for instance in the practice of a prominent local physician, several children in a wealthy family were suddenly seized with severe amebic dysentery. There was no history of suspicious food or water and the children had not been out of the sanitated area. Upon examining the servants for a possible carrier, one maid was found who was passing cysts of E. histolytica in large numbers. Flies are also looked upon as potential carriers and no doubt are responsible for the transmission of many cases. Another prolific source of infection is the Chinese gardener with his luxurious green vegetables, made so by the daily dipper of night soil. We must regard all green vegetables as infected and as a rule one does not eat local vegetables uncooked. I know of no means whereby one can rid them of the potential danger of being harborers of amebic cysts and at the same time preserve the palatability of the article.

In striking at the root of this problem then, we must hunt down the carrier just as we do in typhoid and kindred infections, and we must consider the examination of all food handlers in endemic centers and it is felt that this point cannot be overemphasized. You will find the lower class native to be filthy and careless, this individual may be symptomless himself but a potential source of danger to those about him. Of course, everyone who

ingests cysts does not become infected as was proved by the classical experiments of Walker and Sellards at Bilibid Prison in Manila. Were such the case, everyone residing in the endemic centers would probably be infected as one has but to see the toilet facilities in the outlying districts to realize that obtaining particles of cyst-containing material along with one's food is far from impossible. A recent survey of the waiters at Santa Marta Columbia Hospital of the United Fruit Company, showed 60 per cent infected. At this time it is deemed desirable to emphasize a few important points readily recognized by tropical workers but as a class given scant attention by writers on general medical subjects.

First: Any dysentery may be of amebic origin but we recognize about thirteen other etiologic factors and the task of their differentiation falls to the lot of the laboratory worker in most instances.

Second: That amebiasis may exist without dysentery. That constipation may be present instead of diarrhea and that the infestation may only make itself known by vague gastrointestinal symptoms. The point that I wish to especially emphasize here is that dysentery is *not* the commonest manifestation of intestinal amebiasis.

In the acute cases with the active ameba the diagnosis will be easy and the ordinary examination will readily detect the organism but in the chronic and carrier state other means must be resorted to. The stained specimen of stool offers the only solution to this problem. The technic can be obtained from any textbook on protozoology but the technical work is not easy and the differentiation of the cysts is fraught with difficulty. We are gradually awakening to the fact that we have been heretofore missing from 10 to 40 per cent of our cases for the very reason that we were not making these wet-fixed and stained preparations. Time does not permit me to go into the life cycle of the organism or methods of differentiation.

Another point of considerable importance to be emphasized is the fact that intestinal amebiasis is a protean disease, manifesting itself in many ways. Down in the tropics it is well said that given a case of a vague condition of any kind where one has reason to suspect exposure to amebic infection that condition must be included or excluded before a final opinion is given. And as has been stated before, all residents of endemic centers are exposed to infectious influences as were also many of our soldiers who served in France. It is well within the bounds of possibility that many cases are being passed without being diagnosed in parts of the country where only a rare case is seen. They are frequently missed down there where everyone should be suspected. One may often find a small liver abscess responsible for fever of unknown etiology, the liver abscess being too small to detect by ordinary methods of physical examination. Vague cerebral symptoms may be due to amebic abscess of the brain and an amebic typhlitis can accurately mimic an acute appendicitis. A lung abscess secondary to a liver abscess can closely simulate lobar pneumonia and a failure to make a prompt diagnosis will cost a life.

In examining material from liver abscesses we have found that only rarely are we successful in locating the ameba in the first fluid aspirated from the abscess or from the first gush of pus after opening. The amebae are

not free in the pus but imbedded in the walls of the abscess and can be found in the discharge several days after opening. We have found it indeed poor policy to scrape the abscess wall to obtain material for diagnostic or other purposes as for some at present unaccounted for reason, the slightest manipulation of the abscess cavity has been followed by grave results and usually death. We do not swab out the cavities any more but are satisfied with merely opening and draining.

I promised you in the beginning that this article would be as brief as would be consistent with conveying to you a few practical chapters from our experience. Therefore, I have purposely omitted any reference to microscopic pathology or morbid anatomy. These can be found in a text on tropical medicine.

From the extensive damage done in some of these intestinal cases, one must at once be impressed that those cases that go on to recovery must have suffered so much damage that it would not be possible to bring about a cure in a short time. There may be a symptomatic cure but we must follow up these cases and make repeated examinations to determine that they are free from organisms. Before closing, permit me to stress another point; that is, a suspected case may have to be examined several times before the organisms are found as our approximate statistics show that in only 64 per cent of the positive cases was the first examination successful. According to the best authorities, we will miss from 10 to 40 per cent of our latent cases if we do not resort to the wet-fixed, stained preparations.

CONCLUSIONS

1. This is not meant as an exhaustive treatise upon the subject presented, but merely an effort to summarize some of the work that we are now doing and to demonstrate what has been done.

2. That improved sanitation has been responsible for a sharp decline in the number of cases of amebiasis.

3. That intestinal amebiasis is no longer to be considered as a purely tropical disease, but rather one of a cosmopolitan character.

4. That dysentery is not at all a necessary accompaniment of intestinal amebiasis and it is in the vague case without dysentery that the most skill in examination of the stools is required.

5. That there is a carrier problem and that it is a vital factor in the spread of the disease.

6. That amebiasis is an extremely protean disease and every case of vague gastrointestinal upset must be looked upon with suspicion.

Finally, I wish at this time to express my appreciation for the aid given me by all of the physicians on the Canal Zone for their assistance in compiling this data. To the pioneers I am extremely grateful for without their work this record of improvement could never have been.

DISCUSSION

Dr. Isaac J. Jones.—I don't know that I have anything to offer that would be of any value but I am likewise interested in this subject. I lived in the tropics for fifteen years and spent ten months on the Isthmus itself. There was one statement that rather surprised

me; that is that amebiasis is not ordinarily a water-borne disease. I had one experience personally. In investigating an epidemic of amebic dysentery the people obtained their water supply from a spring at the edge of the town. There was no possibility of an infection from the spring from watershed. In observing the place I found that the women of the town came there to get water; they were all barefoot. They would walk up to the edge of the spring and draw the bucket through the water, and as the bucket was drawn out the water would wash out over the feet and flow back into the spring.

Dr. Kenneth M. Lynch.—Undoubtedly the prevalence of amebiasis varies in different regions, probably with the sanitary conditions. In South Carolina I found more than I do in Texas. The only active cases I have seen in Texas have been imported from Mexico or Central America. The local cases have been inactive or carriers. There is one proposition I want to recall in connection with the spread of the disease: the possibility of the rat as a carrier. I was able to show that such could be the case and that it actually happened, at least in one focus, in 1915. This has been amply confirmed in an experimental way and should be kept in mind.

The second question which I want to discuss is that of the possible and probable common mistake in diagnosis. I have not been so concerned about the reported incidence of histolytica being too low but, rather, too high. It is around one per cent in this region in over a thousand cases I have studied. It is easy enough to mistake coli in free active stage for histolytica. I have seen any number of examples of this mistake and have come to pay little or no attention to an identification of an intestinal ameba except in the encysted stage. I will not make such a diagnosis when the ameba is in the free active stage but I call for a formed stool and wait for the cysts to appear. A fresh stool is not necessary in the identification of amebas, a fresh warm purged stool leads to mistakes instead. You must find the cyst or your diagnosis must be questioned, except, of course, in the case of clinical dysentery, when a working diagnosis can be made very properly.

Dr. A. H. Sanford.—The incidence of amebiasis as reported at the Mayo Clinic may be the subject of some comment. I appreciate that there is considerable difficulty in differentiating between *E. coli* and *E. histolytica*.

The incidence of histolytica in our reports may be too high. It must be remembered, however, that stool examinations are not made routinely. Only those patients with intestinal symptoms are sent to the laboratory for this examination. This would account to a great extent for the high proportion of findings of amebae of the pathogenic type. Since 1919 Dr. Magath, a trained parasitologist, has been studying this problem, and it is not settled yet. There has been a great deal of controversy concerning amebiasis, and there will be still more before the truth is known. Regarding Dr. Feder's contention that diagnoses should be made only with stained fixed wet specimens, I would admit that he may be right. However, amebae are usually found by examining the fresh stool directly with a cover-glass preparation, and a tentative diagnosis made. The one thing that I do want to emphasize is that the men who are here from the north should get busy and examine stools; we have amebiasis in the northern states.

Dr. W. S. Thomas.—Within the last three years we have examined six thousand specimens of stool for protozoa. Eleven hundred of these were inhabitants of New York State and in these cysts of E. histolytica were found in 1:6 per cent. None had symptoms of amebic disease. On account of the low figure and the complete absence of symptoms in the patients harboring the cysts we have refused to become excited about amebic disease in the northern states, in spite of the fact that an occasional case undoubtedly occurs.

Dr. T. C. Terrell.—I think a great deal has to do with the specimen being properly collected. Where possible, we have the patient come to the laboratory and take a good big dose of salts and remain at the laboratory until satisfactory specimens are obtained. We collect at least the first and second specimens and more if necessary, thereby giving us the opportunity of studying both the formed and liquid stools, as the first stool is usually at least partially formed. Of course in those cases where the salts is not necessary we do not put them through that routine.

A PHOTOGRAPHIC METHOD OF COUNTING BLOOD CELLS*

By Arthur H. Sanford, M.D., Rochester, Minn.

THE computation of the cellular elements of the blood has been a clinical laboratory procedure for fifty years. From time to time refinements in methods of technic have been added, but we think of Gowers,[2] Hayem,[4] Lyon and Thoma[5] and others as having established the value of hemocytometry, and of having pointed out some of the inherent difficulties in the procedure. The normal count of erythrocytes was early established at 5,000,000 for the normal adult male.

The usual textbook method for blood counts, also advocated by the investigators of fifty years ago, involves the making of several counts and determining the average. While this procedure is ideal, in practice it is often neglected when the volume of work is such that time is an essential factor, or when the results are normal, or what was expected. There are numerous chances for slight error in as simple a procedure as an erythrocyte count. Various attempts at evaluating the probable error have usually placed it at about 2 or 3 per cent, and always less than 5 per cent. These errors may be due to faulty pipettes, faulty counting-chambers, faulty technic in taking the blood, in drawing it into the pipette, or in making the dilution or in filling the chamber. Recognition of all of these sources of error was responsible for many of the improvements in apparatus.

Another source of error, however, may be in the actual counting of cells as seen in the microscopic field. While the average person should easily learn the technic of counting blood, the fact still remains that there is a considerable personal error in counting up to 500 cells in eighty small squares when the dilution of blood is 1:200. In order to reduce this error to a minimum and to have a permanent record of a count, the photographic method was devised. This method has not been recommended for routine work, although there is no reason why it could not be used as a routine if it should seem advisable.

The apparatus (Fig. 1) was readily assembled. It was necessary to keep the counting-chamber horizontal, and desirable to have a strong light below the stage. The Spencer microprojector, Number 9100, was found to be most suitable for this purpose. This was fastened securely to the framework constructed in the manner illustrated, and an ordinary view camera with a draw of fifteen inches suspended above the microscope of the projector. The total distance from the stage to the back of the camera is twenty-four inches. The eyepiece 6x used with an 8 mm. objective and this camera draw gave a good field of more than 100 small squares of the counting chamber. The magnifica-

*Read before the Fifth Annual Convention of the American Society of Clinical Pathologists, Dallas, Texas, April 15, 16 and 17, 1926.

From the Section on Clinical Pathology, Mayo Clinic, Rochester, Minnesota.

tion is about 200. The negatives are made on process films and prints made, or the prints can be made directly on photostat paper. Two seconds was found to be the optimum exposure for either type of picture.

The counting-apparatus selected (Fig. 2) was a Veeder magnetic counter, form UM, operating on 110 volts direct current. This counter may be obtained wound for six volts and operated by a storage battery. It must operate on direct current.

It was desired to mark every cell after counting it, and to facilitate this the handle containing the "make and break" apparatus (contact stylus, Fig.

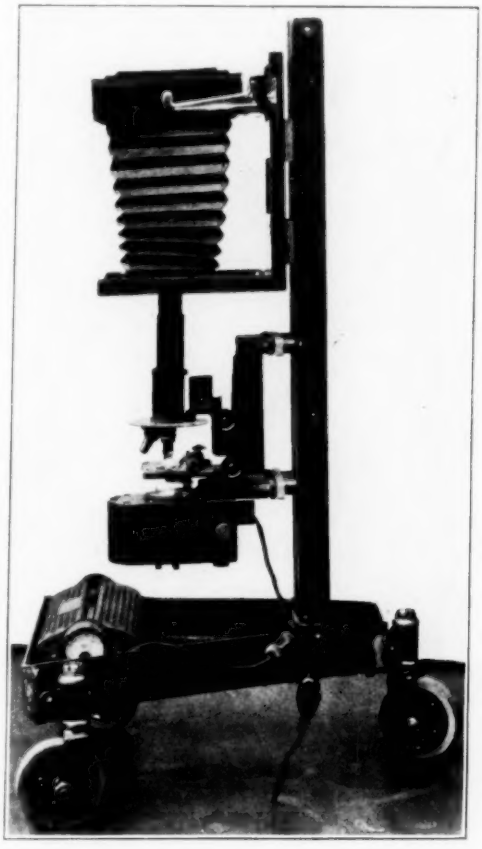

Fig. 1.—Photographic apparatus.

3) was devised by the Mayo Clinic mechanic, George Little. Mr. Little's description of this very essential part of the apparatus is as follows:

"The fiber sleeve *A* is threaded at the ends to receive the terminals *G* and *H* and contains the bakelite plunger *B* and block *K*, *H* being threaded to receive the socket bushing *I*, through which passes cord *J*. The plunger *B* is limited in movement and retained in position by screw pin *D* in slot *C*. *F* is medium-tone steel victrola needle driven into the plunger-terminal *E*. *E* is pushed outward by the coil spring, and, being threaded into the lower end of

plunger *B*, serves to keep *B* down against the screw pin *D*, and to keep current terminals, or switch points, *L*, *M* and *N* open. Point *L* is secured to one cord terminal as shown and *N* to the other. Bringing point *F* in contact with the paper and pushing lightly downward, causes sleeve *A* to carry block *K* down until contact is made with *L*, *M* and *N*, thus closing the circuit and causing the magnet to function and operate the counting-device."

Fig. 2.—Automatic counter.

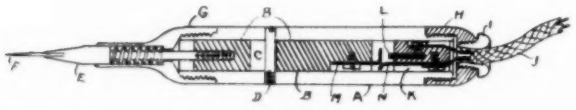

Fig. 3.—Contact stylus.

Several series of counts have now been made with this apparatus. A typical count is illustrated in Fig. 4. These counts have been compared with those made in the usual manner, and it appears that the average technician does not ordinarily count quite all of the cells in the field as registered on the photographic plate. This can be explained in various ways, but possibly the result of constant focusing that a microscopist practices in his effort to bring everything in the field into sharp focus, is that a few cells in the

field are missed by being thrown out of focus. The image of all the cells is registered on the photographic emulsion, however, even if they are not quite in focus.

While this method of counting erythrocytes is original as far as I am concerned, the idea is not new. The title of Amory's[1] paper on "Experiments and Clinical Observations on the Hematinic Properties of Dialyzed Iron," published nearly fifty years ago, would not suggest the manner of making the blood counts, but the beautiful heliotype illustrations accompanying the paper (Fig. 5) excite immediate attention. The author says: "The individual or personal error of vision which is associated with all optical instruments is perhaps somewhat difficult to reduce to mathematic accuracy, on account of the fact that constant observation fatigues the eyesight, and

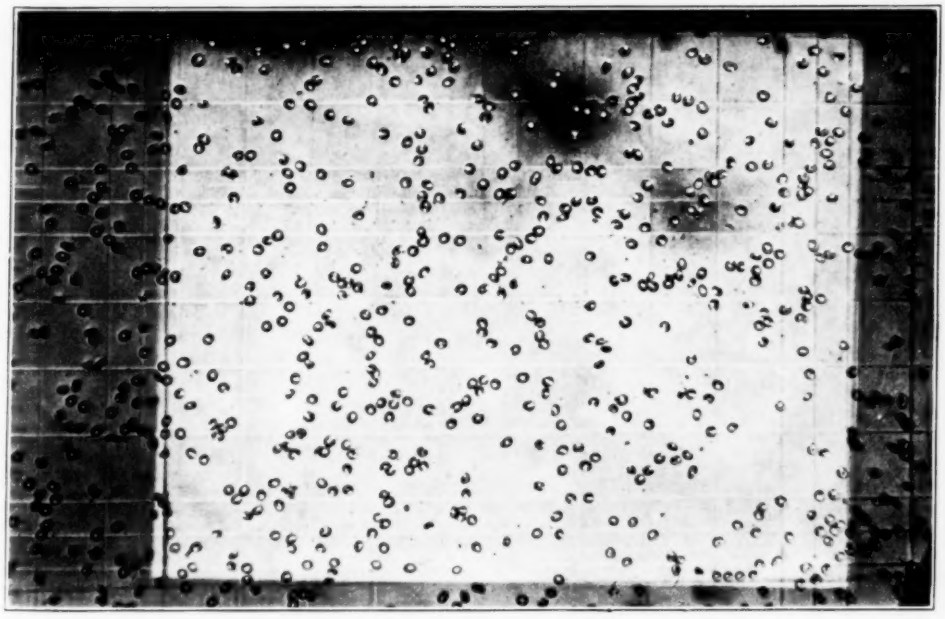

Fig. 4.—Erythrocyte count, showing cells punched out in eighty squares.

hence the results of a series of these observations are subject to an inconstant variation. In consequence of this apparent difficulty, I decided to project upon a photographic plate the image of the corpuscles on the ruled slide, then to print from the negatives, and count upon the print the number of these corpuscles, each one being obliterated as soon as counted."

Reference should also be made to a similar method used more recently by Hardesty[2] in studying the number and arrangement of the fibers forming the spinal nerves of the frog. "In order to count a given section, a photograph of it was fastened upon a small board of soft wood. An automatic registering machine, one common use of which is to count telegraph poles, was modified by attaching to its finger press a short steel rod. Into the end of this rod was inserted a needle." The fibers were punched out on the photograph with the needle and the count registered.

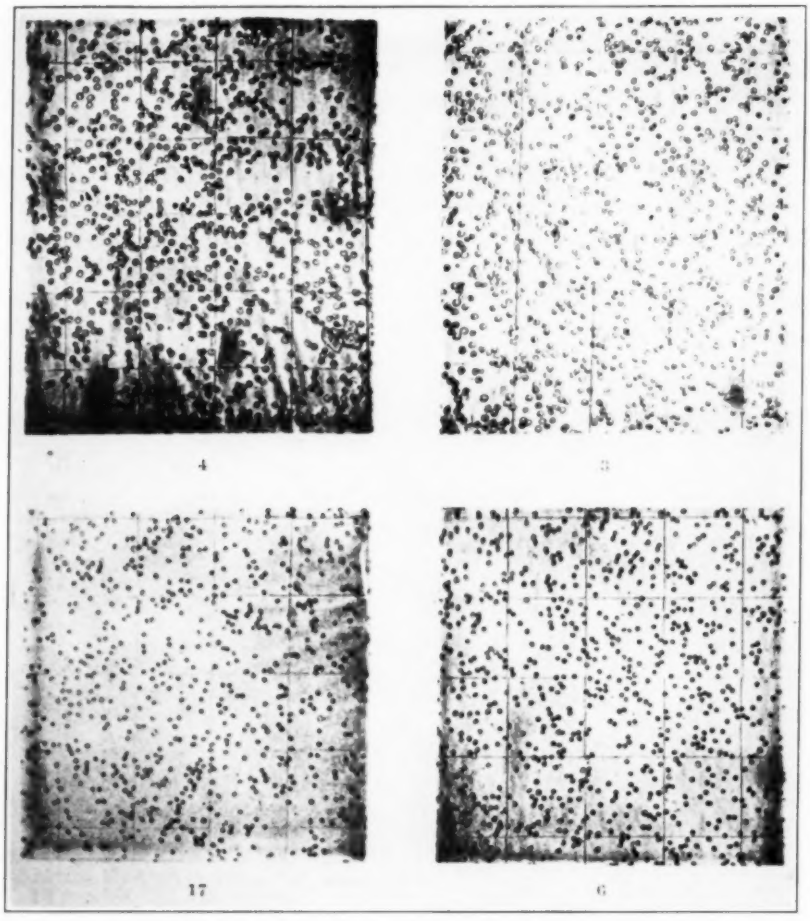

Fig. 5.—Copy of photograph by Amory.

Thus fortified by the arguments of half a century ago, I offer anew a photographic method for counting blood cells.

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DISCUSSION

Dr. H. J. Corper.—I have felt for a long time that we should be taking greater advantage of photographic methods in clinical pathology. I appreciate Dr. Sanford's timely report and compliment him on it. I have been interested in photographic methods for some time and believe the time is coming when we will be urged to utilize photography wherever possible. There is a decided advantage in putting photographic copies in the clinical records

as is now common with x ray pictures. Some day we will be putting into clinical records not only blood counts, but photographs of pathologic specimens as well. When you figure it up financially it is not expensive to use ordinary bromide paper. Direct photographs on bromide paper can go into the records at an average cost of a few cents per copy and are less susceptible of personal error.

Dr. Wm. G. Exton.—I was glad Dr. Sanford brought up this method. We have been doing our blood counts by photography for about ten years using the little instrument called the euscope. You can get the whole four hundred squares. The last six or seven years we have been using ordinary bromide paper. I can say from our experience that the work does not cost as much and is certainly more accurate than the usual counts. With photographic methods, you will be surprised with what little experience you can adopt them to your use.

Dr. A. H. Sanford (closing).—I don't want you to get the impression that this is our routine method. Dr. Corper's suggestion is good. Some day this may be our routine. I am convinced, however, that for research purposes it is worth while and that is what we are using it for at present.

OCHRONOSIS*

By Ernest Scott, M.D., and Robert A. Moore, B.A., Columbus, Ohio

Virchow[1] in 1866 reported a postmortem on a sixty-seven-year-old man, in whom he found that practically the entire cartilaginous system of the body was a coal black, "als ob sie geradezu in gewohnliche Tinte eingetaucht worden waren." He called this condition "ochronosis." Virchow, working with Kuhne, after some chemical investigation, concluded that the pigment was a derivative of hemoglobin and probably was only an extreme degree of a similar pigmentation noted by him previously in old people, and especially lueties.

Since Virchow's original report there have been reported some fifty-three cases of this disease. In the case about to be reported, the fifth, sixth and seventh costal cartilages of the right side were an intense black color. Despite the lack of external pigmentation and the finding of an apparently normal urine, there is evidence that this case is one of true ochronosis. Briefly this evidence is: first, the typical gross and microscopic appearance of ochronosis in the examined cartilages, and second, a pigment which chemically is iron free, exhibits solubilities and staining reactions similar to the melanins, is bleached by sunlight, and gives no spectral absorption.

Case Report.—R. T., fifty-two years old, white woman, married, was first admitted to Mt. Carmel Hospital, May 23, 1922, on the service of Doctor C. S. Hamilton. Six months previously she had noticed a small lump in the right breast. She had not noticed any loss of weight or strength and had never suffered from any serious illness. There was no history of surgical operations. There had been no pregnancies and she passed the menopause four years previously. The patient denied the use of any drug containing phenol.

Physical Examination.—The examination was essentially negative, except for a tumor about the size of a walnut in the central part of the right breast. The axillary nodes were

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From the Department of Pathology, Ohio State University, Columbus, Ohio.

not palpable. The blood pressure was 135/80. There was no abnormal pigmentation of any part of the body.

Laboratory Tests.—Erythrocytes, 5,200,000; leucocytes, 5,200; polymorphonuclears, 35 per cent; small lymphocytes, 55 per cent; large lymphocytes, 10 per cent; eosinophiles, 0 per cent. The urine on numerous occasions showed a pyuria with a trace of albumin, but was not dark in color, nor did it turn dark on standing.

Course.—The right breast was removed together with two-thirds of the pectoralis major muscle and the axillary glands, May 24, 1922. It was noted at the operation that one axillary node was the size of a bean. Microscopic examination showed the tumor to be a scirrhous carcinoma. At the time of this operation no abnormality of the ribs was noted. The wound became infected and a sinus developed. From this, as well as from the blood, a pure culture of a hemolytic streptococcus was isolated. About July 1, 1922, the patient developed definite signs of an arthritis, involving particularly all the joints of the lower extremities. A month later the patient was discharged with an area the size of a dollar which was still draining and covered with granulation tissue. On October 3, 1922, she returned with a diffuse induration of the left breast, which was immediately relieved on curetting the sinus on the right chest wall, and the patient was again discharged. On January 11, 1923, she returned for a thorough curettage of the sinus. At this operation, the following operative notes were made, "Sinus is over the sixth rib in the nipple line, in the center of an area of red scar of half-dollar size. In making a sufficient incision (eventually five inches outward from the parasternal line) it is found that the fifth, sixth and seventh costal cartilages are converted into a black substance, resembling coal. A portion of rib about one and one-half inches long in the immediate neighborhood of the sinus is deprived of periosteum. There is no pus. The black ribs are removed." It was noted also that the costal cartilages above and below the black ones were not noticeably discolored. The patient at this time showed evidence of a definite arthritis of the larger joints. The sinus cleared up and the patient was discharged. A year later she developed symptoms of an obstructive jaundice and died February 9, 1924. A postmortem was not made. For the year following the finding of the black cartilages, the patient showed no abnormal pigmentation and the urine presented no abnormalities.

Pathologic Examination.—Section of the costal cartilages showed the hyaline matrix to be of a diffuse yellow-brown color without granular pigment while the cartilage cells here and there showed some amorphous granular pigmentation. The perichondrium was not pigmented.

Chemical Investigation.—The pigment was soluble by boiling in 0.2 per cent NaOH and was soluble in cold strong acids. Microchemical reactions for iron were negative. Solutions of the pigment when placed in the spectroscope gave no absorption bands. Sections exposed to the sun were slowly bleached. Because of the small amount of material available, the actual chemical composition of the pigment could not be determined. The urine at no time showed a dark color and it did not turn dark on standing.

The etiology of ochronosis has been the subject of considerable debate, based both on observations on man and experimental animals. Based upon the association of certain urinary findings, we may conveniently divide the reported cases into four classes.

1. Virchow[1] in reporting the first case felt that the condition was due to an imbibition of hemoglobin into the tissues. Nardi[2] reporting nine cases of ochronosis from Italy found that intraarticular injection of homogenous blood into rabbits caused the production of a black pigmentation of the joint cartilages.

2. Albrecht[3] in 1902 reported a case with chemical investigation by Zdarek[4] in which the condition was associated with alkaptonuria. He felt that the homogentisic acid united with the chondroitin sulphuric acid of the

cartilages giving the black pigment. Gross and Allard[5] reported a similar case with complete pathologic report by Landois[6] and have subjected this theory to experimentation. They[7] found that if cartilage is placed in a neutral solution of homogentisic acid, in the course of a month it will turn black and is indistinguishable histologically from true ochronosis.

3. In 1906 Pick[8] observed a case of ochronosis associated with the prolonged use of phenol. He reviewed the literature up to that time and came to the conclusion that the condition was due to the deposition of a melanin derived from the oxidation of aromatic compounds such as phenol, homogentisic acid, etc. Poulsen,[9] after an extensive study of nine cases with necropsy on two, came to the same conclusion and proposed that the oxidation was carried out by the enzyme tyosinase, in the same manner formulated by von Furth and his coworkers for the animal melanins in general. Beddard[10] and later, Beddard and Plumtree,[11] are very emphatic in their conception that the use of phenol by some of these cases is of more than passing importance, and cite the fact that one of their patients showed a decrease, if not disappearance of the pigment, on discontinuing the use of phenol. On the contrary, von Amstel[12] feels that the use of phenol by these patients is only a coincidence, but he does think that alkaptonuria and ochronosis bear some relation to each other. Gross[13] has attempted to produce ochronosis by the daily injection of phenol for one year into a dog and calf. They report entirely negative results. Analysis of the time interval in the human cases between the initial use of phenol and the first appearance of the pigment, shows a notable lack of accurate data, yet in a few cases we have figures of three years, four years and one year.

4. In addition to these well-defined cases showing alkaptonuria or giving a history of the continued use of phenol there are certain cases as those of Hecker and Wolf[14] and Poulsen's third case, and the more recent case of Oppenheimer and Kline[15] with chemical investigation by Janney[16] in which a definitely proved melanuria has been present.

5. There are still other cases, notably that of von Hansemann,[17] Harston and Soltan[18] and the present case in which the urine was apparently normal. There are numerous cases including those of Virchow,[1] Heile's two cases[19] and others in which a urinary examination was not made.

Most of the reported cases show a rather general pigmentation of the cartilaginous system of the body. Yet there are several cases, notably, that of Albrecht[3] and that of Ogden[20] as commented on by Osler[21] in which the external pigmentation was limited to the ears. The present case is of this type of localized ochronosis, the pigment being localized as far as is known, to the right costal cartilages, particularly the fifth, sixth and seventh.

An analysis of the reported cases shows the disease to be about equally distributed in the two sexes and that it has occurred as early as twenty-three years[22] and as late as eighty-five years[23] with an average of about fifty years. An analysis of the associated diseases shows no one disease to be of such frequent occurrence as to be of etiologic significance, yet one is impressed by the high incidence of chronic lesions of the joints and cardio-

vascular system. Soderbergh[24] has expressed the opinion that all of these lesions are the result of the same cause as ochronosis, viz., a metabolic disorder, and not the cause per se. The fact that the present case presented the development of an arthritis and pigmentation of the costal cartilages simultaneously gives some support to this view.

In only one case do we find a thorough chemical investigation of the pure pigment, this work being reported by Janney.[16] He found on organic combustion analysis that the pigment has a constitution very similar to that of the melanins reported by Morner. All authors agree that the pigment is spectroscopically inactive.

From this review it seems apparent, that the condition of ochronosis may occur with several associated conditions, to which has been attached an etiologic significance. The proof of the real significance of these associated conditions must be solved by the clinical pathologist, especially by a thorough examination of the urine and a chemical investigation of the pure pigment similar to the recently reported work of Janney.

SUMMARY

1. A case of ochronosis in which the known pigmentation occurred only in three of the right costal cartilages is reported.

2. The condition was associated with a chronic streptococcus infection and arthritis, subsequent to amputation of the breast for malignant disease. The relationship of the infection and ochronosis is problematic.

3. The views concerning the etiology of the condition are reviewed.

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CLINICAL RESULTS WITH PATHOGEN*

By Otto Lowy, M.D., Newark, N. J.†

VACCINE therapy had received several severe setbacks in the past because we did not utilize the fact that in order to be of value the vaccine used must consist of the identical organism which is causing the infection. While we have known that organisms morphologic and culturally are alike and yet different, we failed until recently, to properly differentiate between the various strains of these bacteria.

It is an established fact that infections, particularly focal infections may give rise to symptoms in remote parts of the body. Here again we were confronted by our lack of properly identifying the offending organism. The work of Heist, Lacy, Solis-Cohen, and others have contributed largely toward clarifying some of our difficulties. Briefly stated: They have found that the whole coagulable blood of normal individuals contains immune bodies which prevent the growth of pathogenic organisms, normally found on the various mucous membranes. They have also found that if an individual is deficient in immune bodies against certain bacteria, these bacteria grow luxuriantly in the individual's whole blood.

Burbank and Hadjpolis attack the problem from another angle. They make use of the well-known complement-fixation test, using as the antigen various strains of streptococci, staphylococci, gonococci. They also utilize the native complement of the individual.

This method presents a number of technical difficulties as for instance the preparation and identification of the antigens, furthermore, there are a multiplicity of tests that have to be made in order to arrive at the proper classification. Burbank had been using twenty antigens. After two years work I have succeeded in preparing twelve antigens, each antigen representing a different strain of streptococci.

When we turn to the clinical application of the two methods, we find that the pathogen method is by far the simpler and may even be considered as more accurate. Should, however, the source of infection be in locations such as the frontal sinus, the middle ear or some other inaccessible portion of the body, it would follow that the pathogen method could not be used, and in that event I believe we could fall back upon the complement fixation.

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†Serologist to Newark Beth Israel Hospital.

Experimental.—In order to determine what analogy if any, existed between the two methods, we examined five cases of chronic arthritis without deformity, by means of the pathogen method and found Streptococci viridans in four cases and a nonhemolytic streptococcus in one case. These organisms were grown in pure culture. One portion of the growth was utilized for the preparation of an antigen and the other portion reinoculated in the patient's own blood.

Results.—Complement fixations were positive in varying degrees with all antigens. The pathogen showed growths in all instances. When the complement-fixation test was applied by using antigen one, against blood two, etc., we found that three of the strains gave positive reactions, which would seem to indicate that these strains were identical with one another.

*Treatment of Patients with Pathogen.**—Ten cases of subacute and chronic arthritis, three cases of chronic bronchial asthma, were treated. Growths were obtained from either nose, throat, teeth and sputum. It may be noted that in the asthma cases plain broth cultures showed a large number of gram-positive and gram-negative organisms, whereas, using the whole blood only streptococci and pneumococci, were isolated. The vaccines were prepared and injections given at three-day intervals, starting with fifty million and increasing the dose gradually, going as high as one billion.

Reactions.—Reactions with the exceptions of slight soreness at the site of injections were not observed.

Results.—Four cases of subacute type of arthritis were completely relieved of their symptoms, two improved and four unimproved, of the asthma cases one improved, two unimproved.

Check Up.—Up to date I have been able to check up only on two cases by again taking culture and attempting to grow them in the patient's own blood. Both of these were cases that had been improved and in both instances we were still able to demonstrate the streptococci in the growth.

CONCLUSIONS

1. Vaccine therapy in focal infections is of value only when the strain causing the infection is used for purpose of preparing vaccine.

2. Individuals suffering from focal infections should have the foci of infection removed.

3. Both the pathogen and complement-fixation tests are of value in determining the organisms.

DISCUSSION

Dr. George T. Caldwell.—I am of the opinion that organisms do not grow in whole blood but are able to remain alive.

Dr. O. Lowy (closing).—I feel that any method which gives promise of being accurate and which gives results is worth while looking into.

*Clinical material obtained from the service of Dr. Szerlip, Newark Beth Israel Hospital.

THE TREATMENT OF ONE HUNDRED FIVE CASES OF ACID INTOXICATION WITH BUFFER SOLUTIONS*

By F. A. Hecker, M.D., Ottumwa, Iowa

A REVIEW of the literature on acidosis or perhaps better stated acid intoxication at this time is extensive and the theories offered of the etiology are many and diverse. It is believed that acid intoxication is due to the accumulation of acid products in the blood which are due to faulty elimination which results in disturbed metabolism.

With our present knowledge acid intoxication may be divided into two groups.

Group 1. This group clinically is characterized by dry skin, dry tongue, air hunger and in the terminal stage by coma. The clinical symptoms it is believed are due to toxic substances which are the result of metabolic changes which deplete the tissues of their buffers.

Group 2. This group it is believed is due to toxic substances produced by the growth of bacteria in the tissues which they have invaded. By the absorption of the toxic substances an impaired chemical balance of the blood occurs. Consequently the vital organs and the tissues of the body are depleted in their buffers.

These two groups present chemical changes in the blood, the expired air, the alveolar air, and the urine.

From the foregoing, we are taught the blood of persons affected by acid intoxication shows a depletion of the blood buffers, namely, the sodium bicarbonate and the disodium phosphate. In addition to the blood buffers the calcium, and the magnesium are also diminished in quantity when compared to the quantity normally present in the blood. When this chemical imbalance occurs it can be corrected by administering the needed salts by mouth or by introducing them intravenously.

The first method employed for the determination of acid intoxication was the carbon dioxide capacity of the plasma of Van Slyke and Cullen. This method was also employed for the determination of the progress of the treatment. As several specimens of blood were needed for the determination of the progress of the treatment, we were confronted by objection by the relative of the patient. All of the patients treated were pay patients. Hence we were compelled to comply with the wish of the relative or discontinue the work. It is for this reason that this excellent method was discontinued.

The next method tried was the alveolar carbon dioxide tension method of Fridericia. Employing this method one must have the cooperation of the

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From the Laboratory of Clinical Pathology, St. Joseph Hospital, Ottumwa, Ia.

patient. This at times is difficult if not impossible to obtain if the patient is in a semicomatose condition or is desperately ill. Hence this method was discontinued.

The next method tried was the determination of the P_H of the urine with the hydrogen-ion apparatus. The specimen of urine was collected volitionally or with the catheter. All specimens of urine collected were sent to the laboratory in chemically clean, tightly stoppered bottles and were stored in the ice chest until titered. With this method the objections offered in the two foregoing methods were overcome and was the method selected for the determination of acid intoxication and the progress of the treatment.

The Michaelis Fraction.—The observed H-ion concentration of the urine.

$$\frac{\text{Primary Phosphates}}{\text{Secondary Phosphates}} = \frac{H}{2 \times 10\text{–}7}$$

$2 \times 10\text{–}8$
$2 \times 10\text{–}7$
$2 \times 10\text{–}6$
$2 \times 10\text{–}5$

I considered a urine having a P_H of 5.00 or less as indicative of acid intoxication.

The following glassware is employed. An all-glass still; a two-way glass stopcock; an all glass twenty c.c. syringe; the barrel of a one c.c. syringe which has been pulled down to one-fourth of an inch in diameter and three, five hundred c.c. Erlenmeyer flasks. All glassware and rubber tubing must be chemically clean.

A good method for the distillation of water is as follows: 800 c.c. water is poured into the flask of the still, after which a few crystals of potassium permanganate are added and the distillation is begun. The first 100 c.c. water coming over is collected in one of the Erlenmeyer flasks and used for rinsing them. One of the flasks is placed under the outlet of the still and the remaining two flasks are stoppered with gauze. As soon as 500 c.c. water has been collected it is equally divided in the stoppered flasks. To one of the flasks is added 4.25 gm. of sodium chloride C.P.

The two flasks containing the water and the stopcock and accessories wrapped in a clean towel are now placed in the autoclave and sterilized. When sterilization has been completed the flasks are removed from the autoclave and one of them is cooled in running water until it is comfortably tolerated by the back of the hand. This step is followed by the determination of the dose of the alkali.

The quantity of the dose of the alkali is determined as follows at this time. One-third to one-fourth of the weight in grams per kilogram of body weight in the proportion of two parts of sodium bicarbonate to one part of disodium phosphate minus 4.25 grams of the sodium chloride. The weighed amounts of the sodium bicarbonate and the sodium phosphate are then dissolved in the cooled flask. The contents of the other flask, one-half of the sterile water, is then added to the flask in which the salts have been dissolved. A 10 c.c. sample of this solution is placed in a chemically clean beaker and is titered as follows.

The burette is filled with a 10 per cent solution of the monopotassium phosphate. The beaker is placed on the stand of the hydrogen-ion apparatus. The electrodes are immersed in the solution and the hydrogen electrode charged with hydrogen gas. This step is followed by allowing the solution of monopotassium phosphate to drop into the solution in the beaker. The solution in the beaker is stirred gently while the monopotassium phosphate is dropping into it. After 8 c.c. of the solution in the burette has dropped into the beaker the stopcock of the burette is turned off and a trial titer is made with the hydrogen-ion apparatus. If the bridge reading does not correspond to the P_H of 7.00 the solution in the burette is added and trial titers are made from

Fig. 1.—Assembled apparatus used for giving intravenous buffer solution.

time to time. The moment the bridge reading approaches a P_H of 7.00 the burette stopcock is turned off and a routine titer is made. If the reading is not correct continue the foregoing technic until the desired P_H of 7.00 is attained. This step is followed by reading the burette, from which the volumetric correction can be determined and the 500 c.c. of the solution is buffered with monopotassium phosphate to a P_H of 7.00. The solution is now filtered through sterile cotton.

The stopper supporting the two-way stopcock is pressed firmly into the mouth of the flask containing the buffer solution. The outlet A when the valve of the stopcock is set at B communicates with the glass tubing C which

is immersed in the solution in the flask. The outlet A′ when the valve is set at B′ communicates with the syringe attached to the rubber tubing attached to the outlet A. Hence to operate the apparatus one turns the valve of the stopcock to the position A-B-C to fill the syringe and to the position A′-B′-A to discharge the content of the syringe through the hypodermic needle attached to the glass connection. All air must be removed from the rubber tubing and the glass connection. The flask is now set aside and the blood pressure, pulse, and fever taken. This step is followed by the routine preparation of the site for the intravenous puncture and the intravenous puncture is made. The moment the needle enters the vein, blood appears in the glass connection to which the needle is attached. The next step is that of making gentle pressure with the syringe and if there is no distention of the overlying tissue at which the intravenous puncture has been made the intravenous administration of the solution is begun. The usual time required to give the dose is fifteen to twenty minutes.

The reaction following the intravenous administration of the buffer solution varies. The peak of the alkalinity of the urine varies in time. The chill varies in time, onset, and duration. The severity of the chill varies from a feeling of chilliness to a severe chill. The respiration as a rule is not affected, although in several instances a partial cessation of respiration has occurred for a short time. The fever usually rises rapidly sometimes reaching 107° F. Delirium and restlessness of varying severity sometimes follow the intravenous administration of the dose. In most instances the patient becomes quiet. The sweating usually follows the peak of the fever and the chill.

Immediately after the buffer has been given the patient should be covered with a woolen blanket and then packed in a hot pack to promote sweating, and usually kept for an hour. There are cases however when the hot pack should be continued as a matter of observation following the reaction. The patient should be well covered with a woolen blanket when the hot pack is removed and the wet gown should be replaced with a dry one. At the end of the second hour the blanket is removed and the patient is covered with routine bedding. The rectal temperature should be taken at half-hour intervals for two to four hours after the dose has been given, and then taken every two hours for the remaining twenty-four hours. Many times the recipient of the buffer solution is very thirsty and should be given water freely unless otherwise indicated.

The report offered in this paper was begun in 1923 and since that time 105 cases of all kinds have been treated with the buffered solutions to determine if possible their value in all affections which are accompanied by or followed by acid intoxication. All of the patients treated with the buffered solutions were desperately ill. It is not the purpose of the writer to lead the reader to believe that a panacea for acid intoxication has been found. But the writer does believe that many cases have been helped over the rough spots with the aid of the buffered solutions. The following tabulation shows the success and the failure of treated cases. Of 105 cases treated, 65 recovered and 36 died, 4 showed little or no improvement from the treatment but recovered.

CASES TREATED WITH BUFFERED SOLUTIONS

Diabetes	
Recoveries	4
Deaths	3
Erysipelas	
Recoveries	6
Deaths	1
Eclampsia	
Recoveries	4
Deaths	2
Pneumonia	
Recoveries	4
Deaths	3
Pyelitis	
Recoveries	4
Improvement	1
Streptococcus peritonitis	
Recoveries	0
Deaths	3
Postoperative appendectomy	
Recoveries	9
Deaths	4
Puerperal sepsis	
Recoveries	3
Deaths	1
Puerperal sapremia	
Recoveries	4
Deaths	0
Streptococcus septicemia with endocarditis	
Improved (died later)	1
No improvement	2
Streptococcus septicemia following abortion	
Recoveries	0
Deaths	4
Streptococcus sore throat	
Recoveries	4
Deaths	0
Nephritis, all types	
Recoveries	8
Deaths	5
Hysterectomy	
Recoveries	4
Deaths	2
Cholecystotomy	
Recoveries	2
Deaths	1
Cholecystectomy	
Recoveries	1
Deaths	3

One case vomiting of pregnancy (not improved).

One case pyonephrosis during pregnancy (not improved).

One case streptococcus empyema with pyonephrosis (some improvement).

One case streptococcus septicemia (recovered).

One case pelvic abscess following delivery, pre- and postoperative treatment (recovered).

One case ruptured gall bladder preoperative (recovered).

Toxic exophthalmic goiter preoperative (recovered).

One case scrotal abscess postoperative (died).

One case ruptured pancreas postoperative (recovered).

One case prostatectomy postoperative (recovered).

One case suprapubic drainage postoperative (died).

One case ruptured tube postoperative (recovered).

Before closing I wish to thank the physicians who so kindly permitted me to give buffer solutions to their patients. I also wish to thank Dr. A. Itano formerly of the Massachusetts Agricultural College for the many suggestions when this work was begun.

SUMMARY

Thus far no ill effects have followed the intravenous administration of the buffer solutions.

The buffer solutions are not a panacea for the treatment of acid intoxication. Many times hopeful cases terminate in failure.

Theoretically the buffer solutions as a means for replacing the depleted buffers is correct, provided our present teaching is correct. Namely, that the blood buffers are the sodium bicarbonate and the disodium phosphate.

The titration of the urine with the hydrogen-ion apparatus is not difficult, and is a good method for the determination of acid intoxication and the progress of the treatment. The determination of the dose at this time is not difficult. Making the buffer solution and its administration is simple and quickly performed.

DIABETIC GANGRENE TREATED BY INSULIN*

By Horace Gray, M.D., Santa Barbara, Calif.

UNDER this title DuPré[1] has reported an instance of moist gangrene with ulcer exposing the tendons, "soundly healed" after three months. A similar satisfactory outcome seems worth putting on record, in view of the outright preference for operation on the part of medical men of experience with diabetes. Joslin[2] for example wrote: "One hears of so few recoveries with enjoyment of life after months of medical treatment that I cannot help urging surgery at an early stage"; and Blotner and Fitz "have not been particularly struck by the effect of insulin upon the healing of gangrene once it has developed."[3]

REPORT OF CASE

Mrs. E. J. A., a negro woman, aged sixty-six years and eleven months, 156.2 cm. (61.5 inches) tall, began gradually to be troubled with nocturia and pruritus vulvae about July 1, 1918. Six months later she complained to a doctor, who then found sugar in the urine. Her weight had been greatest in 1914, namely 200 pounds with her clothes, while at the time of diagnosis it was about 185 pounds, and was 167 when last noted, one month before she came to me. This visit was the result of neglect of treatment (other than temporary abstinence from free sugar for a short while after diagnosis), eventuating in the following complaints. "Six weeks ago a gummy discharge on her stocking, then four weeks ago the ball of the right big toe 'peeled off'; but still she wore her shoe and did the work in her own little house alone. Suddenly six days ago she found her foot discolored, especially on top, when she took off her shoes at 10:30 P.M." The next day, January 4, she stayed up around the house, shoeless. Jan. 5 and 6 she spent abed, finally calling a doctor in the evening. A sample of urine contained 2.0 per cent of sugar but no diacetic acid. On Jan. 9, when I first saw her, a specimen contained 2.7 per cent sugar, but no diacetic, then nor later. Her temperature was 100.4°, pulse 112, respiration 20, blood pressure 145/85, white blood count, 24,600. Her right foot was all bluish red, and under the big toe was a sinus oozing only a little pus, while on the dorsum was an ulcer 2.5 cm. wide, foul but semi-inspissated. The lung and other examinations were negative. The Wassermann and blood culture turned out negative. In the office she was given 10 units of insulin and sent into hospital at 5 P.M., where the urine was obtained at intervals of one to two hours, and after each positive test, ten units of insulin were administered subcutaneously. At 5 A.M. the voiding was sugar-free, insulin totaling 50 units. Weight 147 pounds net (66.7 kg.). The insulin was cut to 10 units three times a day a. c., and a diet was given of about 0.5 gm. protein and 15 calories per kilo, which was taught to her on the basis of Joslin's maintenance diet C 7 PF 5, (about carbohydrate 70 grams, protein 35, fat 60, calories 1000). No twenty-four-hour urine thereafter contained more than 13 grams of sugar, and since January 16, the sixth day in hospital, sugar was found only once in the remaining 35 days in hospital and not at all in any of the

*From the Santa Barbara Clinic.

24-hour collections submitted at intervals since, including the latest on Aug. 9. The insulin was gradually reduced to zero twenty days after admission, while still on 1000 calories. The diet was then gradually increased to C 135, P 66, F 92, Cal. 1632, on which she was discharged February 22, 1926.

The foot was of course shown to a surgeon *at her first visit*, and she was watched carefully with a view to probable operation, especially as there was some pain in the foot, which Joslin regards as an indication for operation. X-ray examination by Dr. J. G. Ware showed marked bony absorption and decalcification of the distal phalanges of the right toes, especially of the big toe, also of the distal portion of the proximal phalanx of the big toe; and lesser changes in the toes of the left foot. The treatment at first consisted of hot and cold plunges for ten minutes three times a day (the cold had to be omitted because it increased the pain), followed by dry heat with an electric baker, wet corrosive dressing and elevation of the foot for thirty minutes. Alternate hanging of the foot over the edge of the bed, as in Buerger's exercise cycle, was discontinued because painful. Drainage became free but as the fever decreased only in part, the order was changed on January 15 to *dry* dressings. The next day the temperature fell to normal and remained so. The foot improved very gradually, but there was no setback, so that on February 22 she was allowed to go home though neither ulcer nor sinus was completely healed. Total healing occurred about May 9. Follow-up August 9, seven months after I first saw her, revealed body weight 150 pounds dressed, the twenty-four amount sugar-free, the foot sinus and ulcer both healed, and indeed the second and middle toes capable of a moderate amount of motion dorsally. The patient was found caring not only for herself but for house guests attending a church convention.

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LABORATORY METHODS

THE STABILITY OF CARBOHYDRATE MEDIUMS*

By Lucy Dell Henry, B.Sc., and M. S. Marshall, Ph.D., Lansing, Mich.

THE identification of bacterial species plays an important rôle in the daily routine of a diagnostic laboratory. The means of identification must be unequivocal. When discrepancies occur two possibilities may be considered in locating their source,—the technic (including bacteriologic reagents), and the organism, for no living organism is invariant. For example, if an organism presents many of the ordinary characteristics of a certain species, but its reaction to a certain carbohydrate is eccentric for that species, the carbohydrate medium may be at fault or the organism may be peculiar in this respect. With the primary purpose of determining the conditions under which our carbohydrate mediums were to be relied upon, a series of experiments was made over an extended period. In some respects the work, directed as it was toward practical ends rather than toward fundamental scientific investigation, is obviously superficial. However, the results, briefly stated, may be at least of practical value to other workers as they are to us.

The use of filtration as a means of sterilizing carbohydrate solutions for bacteriologic work, although in all cases not absolutely necessary, is certainly from the standpoint of the integrity of the sugar the only safe procedure. Carbohydrates heated in culture mediums not always neutral in reaction are very likely to undergo some hydrolysis, sometimes to an objectionable degree. This premise has been accepted on the basis of the recommendations of Kendall and his associates[1] pertinent to work in biologic methods of identifying carbohydrates, on the basis of the discrepancies occurring in the literature regarding fermentation reactions, and on the basis of our own experiences with difficulties for which no explanation other than heating of the carbohydrates could be found. There certainly exist occasional variations in the fermentation reactions of some well-defined bacterial species, but one has every right to expect a reasonable degree of stability under comparable conditions.

EXPERIMENTAL

Using seven of the more frequently used carbohydrates, dextrose, lactose, sucrose, levulose, maltose, mannite, and xylose, 20 per cent aqueous solutions of each were prepared and filtered through sterile Mandler filters. These were stored in Pyrex glassware at 5° C. during the experimental period. The medium in which these solutions were tested consisted of beef extract broth,

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made sugar-free by incubation with *B. coli communior* and containing the usual 1.0 per cent peptone and 0.5 per cent NaCl, with 0.5 per cent agar, and Andrade's indicator, the final product having a P_H of 7.1. The carbohydrate concentration was made 0.5 per cent in the melted partially cooled agar. This gives a semisolid base into which inoculations may be made by straight stabs in the center of the tubes. Thirteen cultures were used for checking, all members of the enteric group, and through long culture on artificial medium presumably stabilized: *B. coli communior*, *B. coli communis*, *B. typhosus* (two strains), *B. paratyphosus A*, *B. paratyphosus B*, *B. dysenteriae* (Shiga, Flexner, Mt. Desert, and Hiss Y types), Morgan's bacillus, *B. aerogenes*, and *B. alkaligenes*. During the experimental period these cultures were transferred weekly on beef infusion agar slants.

For a period of one year each carbohydrate was tested weekly in freshly made medium against each of the above cultures. Thereafter the tests were made at uneven intervals up to a period of twenty months from the time of the original filtration of the sugars. At the final testing a duplicate set of sugar mediums was prepared from freshly made 20 per cent sugar solutions, and brom thymol blue was used instead of Andrade's indicator in order more closely to follow and compare the P_H changes. Without attempting to illustrate by the extensive tables necessary to show the details of the reactions of the series, the results may be briefly discussed.

Dextrose is fermented probably by all organisms capable of fermenting any carbohydrate, and, inasmuch as Kendall has shown that very minute amounts of sugar may be demonstrated by biologic tests, a considerable decomposition might have occurred with dextrose without destroying its apparent specificity as a fermentable sugar. Hence, it is not surprising to find that the extended period of storage of the dextrose solution shows no effect as biologically tested. The *B. coli* strains, *B. aerogenes*, Morgan's bacillus, and the paratyphoid cultures produced acid and gas and the other cultures excepting, of course, *B. alkaligenes*, produced acid both at the beginning and at the end of the twenty-months' period.

Lactose, with which more trouble might be expected, was very consistent during the entire period, with the exception of doubtful fermentations on several occasions with Morgan's bacillus and with the four dysentery strains. These reactions are explained by the fact that the sugar medium, made up on scheduled time, was not inoculated until some days later. The sugar solution per se was stable, but the sugar medium, although stored at the same temperature, was not. This has been confirmed by repeated observations, not only with lactose but with other carbohydrates.

Sucrose solution remained stable during the twenty-month period. Several abnormal reactions appeared exactly as in the lactose tests, and on the same dates, due to storage of the medium containing the sugar. The Flexner dysentery strain produced acid from the sucrose solution for the first twenty-seven weeks, and thereafter produced no acid with the exception above noted. In our final test, however, in which freshly prepared sucrose solution as well as the stored one was used, this culture produced alkali in twenty-four hours,

in thirty-one hours acid production had reduced the reaction slightly (P_H 6.8), and in forty-eight hours the acid production was as marked as with any sucrose fermenting culture. Thus time is an important factor in following carbohydrate fermentations. The progressive reactions in the two sets of mediums were, however, exactly parallel, the use of brom thymol blue making possible a detailed record of changes in P_H value.

Maltose offered more difficulties than any other carbohydrate used, although its stability stored in 20 per cent solution was constant so far as biologic tests were concerned. The *B. coli* strains and *B. aerogenes*, the two typhoid strains, the *B. paratyphosus A* strain, and the Flexner and Hiss Y dysentery strains showed a constant reaction during the twenty-month period. Beginning on the seventh week the *B. paratyphosus B* strain, normally producing acid and gas, failed for four consecutive weeks to react. Using fresh carbohydrate solution, three strains originating from the same source were tested against this sugar, one the experimental culture, one which had been kept on similar medium but less frequently transferred, and one which had been kept on a sugar-free semisolid medium. Of these the first gave no fermentation (the same response as given to the stored maltose solution), the second gave some, and the third gave normal acid and gas production. By putting the experimental strain through a generation on sugar-free semisolid agar, the normal fermentation reaction returned, and never again disappeared. The Shiga dysentery strain gave several peculiar reactions, but these appear to be due to the period of incubation of the inoculated tubes. In the final experiment, comparing old with fresh sugar solutions, the results with the Shiga culture were exactly parallel: slight acid in two and one-half hours, definite in four and one-half hours, neutral again from eight to thirty hours, after which there was a very slight trace of acid. The Mt. Desert dysentery culture and Morgan's bacillus gave similar reactions.

Levulose is normally fermented by all cultures of those used except *B. alkaligenes* and showed no change during the twenty months of biologic testing.

Mannite was stable throughout the twenty months. *Xylose* showed no change over a period of twelve months, at which time the supply of filtered sugar was exhausted.

The specific rotation of the sugar solutions was measured after one month, after two months, and after twenty months. The results are not all that might be desired, since the sugars were not specially purified, and storage was under practical rather than under ideal conditions; thus the physical conditions for a high degree of accuracy were not met. The goal was not so much the determination of specific rotation as the securing of a control for our biologic results. With fresh solutions there was no great discrepancy between the rotation of the sugar solutions as measured and tabulated values[2] secured under optimum conditions. After twenty months of storage, however, the specific rotation was from 10 to 40 per cent greater in all of the carbohydrate solutions except mannite and xylose. Mannite does not rotate the plane of polarized light, and did not rotate it after twenty months standing. Xylose

could not be tested because of insufficient amount. Determination of the concentration of sugar in several of the stored solutions showed them to be less than 22 per cent, an increase of less than 2 per cent due to evaporation, so that the considerable increase in rotation could not be explained on this basis.

SUMMARY

Twenty per cent unheated filtered solutions of dextrose, lactose, maltose, sucrose, mannite, and levulose stored at 5° C. appear to retain their specific properties with regard to biologic fermentation for a period of at least twenty months; xylose similarly prepared is stable for at least twelve months. The addition of such solutions to culture medium aseptically, to avoid heating, specifically a beef extract sugar-free semisolid agar, furnishes an excellent means of checking fermentation characteristics of bacteria, but the storage of the medium after the addition of the carbohydrate for more than a few days renders the fermentation reactions nonspecific. Variation in the fermentation of some carbohydrates by some bacterial strains may under unknown circumstances occur, but every possible means of demonstrating that such variation does not occur should be exhausted before accepting such a conclusion. Fermentation reactions should be read, for example, at four, six, eight, twenty-four, and forty-eight hours from the time of inoculation, for it is essential that one view fermentation as a progressive process with several possibilities rather than as a static definitely positive or negative matter.

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A MICROSCOPIC SLIDE PRECIPITATION TEST FOR SYPHILIS*

(SECOND COMMUNICATION)

BY B. S. KLINE, M.D., AND A. M. YOUNG, M.D., CLEVELAND, OHIO

IN A previous paper[1] details of a microscopic slide precipitation test for syphilis with Kahn's antigen dilution were given. In a report (to be published) the results obtained by this method in 2809 tests will be given in detail. In that study it was found that there was agreement of the slide precipitation test with the condition of the patient in 94.9 per cent. The Kahn test and the Wassermann test with an ether insoluble antigen agreed with the condition of the patient in 95.2 per cent. The Wassermann test with an acetone insoluble antigen agreed with the condition of the patient in 93.5 per cent.

*From the Laboratory Department of Mt. Sinai Hospital.
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Although the agreement of the slide precipitation test with the condition of the patient was practically the same in number as that of the Kahn test and a sensitive Wassermann test, its disagreement (in 5 of 100 tests) was more frequently on the basis of a positive reaction in nonluetic serum, whereas the disagreement of the other tests was more frequently due to a negative reaction in luetic serum. It became apparent therefore that the slide precipitation test as performed, although even more sensitive than the Wassermann and the Kahn tests, did not quite equal those tests in specificity. This difficulty has been thoroughly overcome by the use of an antigen completely free of precipitate at room temperature, by shortening the time of the test and especially by more thorough immediate mixture of the antigen dilution and serum. The vast majority of the 2809 tests were done with an antigen containing a fine precipitate at room temperature, dissolved just before use by placing the ampule in hot water. The immediate slide precipitation test as now performed is not only more sensitive than the Wassermann test but just as specific (see Tables I and II). Furthermore, it requires no humidor cover and may be done regardless of the humidity of the room. The temperature, however, should be no less than 70° F. and the glassware and ingredients should not be cold. In addition, the test has been simplified by the use of a microscopic slide 2 by 3 inches holding 12 paraffin rings instead of 3 microscopic slides, 1 by 3 inches, each holding 4 rings.

TABLE I

IMMEDIATE SLIDE PRECIPITATION AND WASSERMANN TESTS WITH CLINICAL COMPARISON

52 Luetic 3 Doubtful 346 Nonluetic sera	*Immediate Slide Precipitation Test* (Antigen clear at room temperature)		*Wassermann Test* (Ether insoluble antigen)	
	Tests	%	Tests	%
Agreement	395	98.5	368	97.35
Disagreement				
a. One test positive, patient nonluetic	2	0.5	3	0.8
b. One test negative, patient luetic	4	1.0	7	1.85
	6	1.5	10	2.65
Total	401		378	

TABLE II

COMPARISON OF IMMEDIATE SLIDE PRECIPITATION AND WASSERMANN TESTS

	MT. SINAI HOSPITAL		CLEVELAND HEALTH DEPARTMENT KINDNESS OF MR. C. REINSTEIN	
	Tests	%	Tests	%
Agreement	364	92.62	409	91.7
Relative Agreement	21	5.34	23	5.6
Agreement or Relative Agreement	385	97.96	432	97.3
Disagreement	8	2.04	14	2.7
Total	393		446	
Wassermann Anticomplementary, Slide Precipitation Test Variable	20	4.8	7	1.5
	413 Done		453 Done	
			866 Slide Precipitation Tests in All	

Evaluation according to the method of Kahn:—
Positive Reaction ++++, +++ and ++.
Doubtful Reaction + and ±.
Agreement=Positive or negative by both methods.
Relative Agreement=Positive or negative by one method and doubtful with the other.

IMMEDIATE SLIDE PRECIPITATION TEST FOR SYPHILIS

Glassware.—Microscopic slides 2 by 3 inches as purchased are rubbed on both sides with bon ami paste (prepared by allowing a cake of bon ami to remain in sufficient warm water to cover it for twelve hours or more). As soon as the paste is dry (in about five minutes), it is completely removed from the slide with a soft muslin cloth. For convenience the slides covered with paste may be stuck to each other, allowed to dry and cleaned at any time. Upon the clean slides, 12 paraffin rings, each with an inside diameter of 11 to 12 mm., are

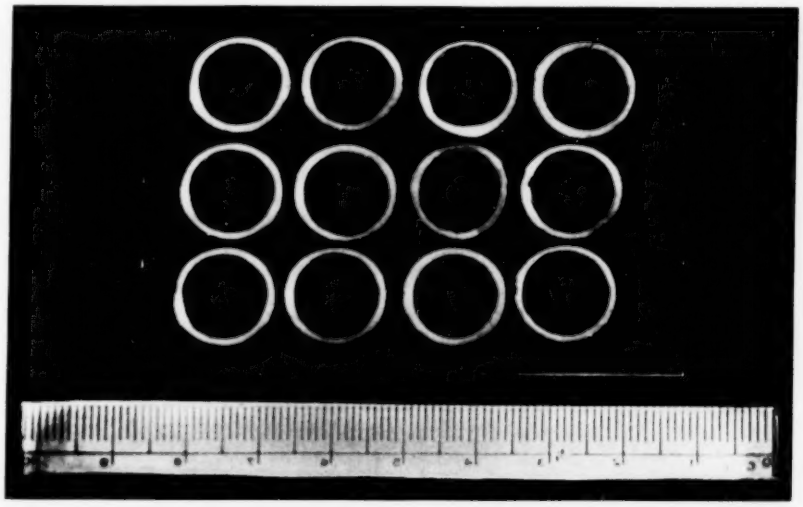

Fig. 1.—Microscopic slide 2 by 3 inches holding twelve paraffin rings (exact size).

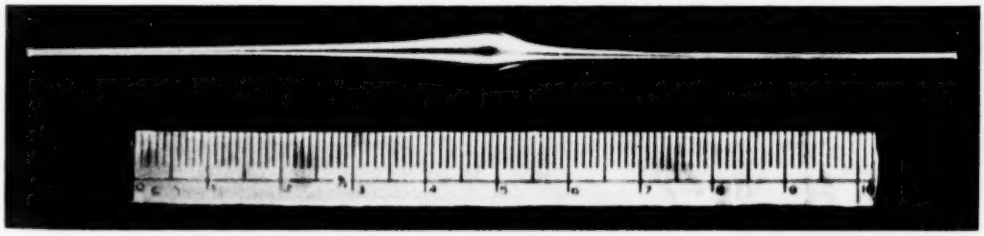

Fig. 2.—Capillary pipette for antigen dilution (exact size).

mounted (see Fig. 1). After use the slides may be washed in hot water and prepared again as outlined above.

Instrument for Making Paraffin Rings.—This is essentially the instrument proposed by Green.[2] A piece of soft iron wire (No. 28) 14 cm. in length is wound twice tightly about a test tube 12½ to 13 mm. in outside diameter, forming a double loop and leaving a double shaft about an inch in length. The two shafts are then twisted together to within a quarter of an inch of the free end. After removing the looped wire from the test tube, a piece of linen thread (No. 12) about a yard long is started from the free end of the shaft after being fastened here by a single twist of the two free ends. Three long

turns are made reaching the loop which is then tightly wound with the thread; the winding is continued up the shaft to the free end where it is fastened between the two ends of the wire by twisting them. The loop is then bent at right angles to the shaft. It is then reshaped by working the loop against the bottom of the test tube mentioned above. The shaft is then inserted into the handle of a teasing needle or into a straight hemostatic forceps.

The paraffin rings are made by dipping the instrument into smoking paraffin (about 120° C.), draining quickly at one point and transferring the remainder to the glass slide.

Pipettes.—The pipettes needed for delivering the sera are the ordinary 1 c.c. pipettes graduated in 0.01 c.c. The pipette for the antigen dilution is a capillary pipette made from glass tubing 6 to 8 mm. in diameter (see Fig. 2) with the tube about ½ mm. in diameter, delivering a drop equal to 0.0075 to 0.0085 c.c.

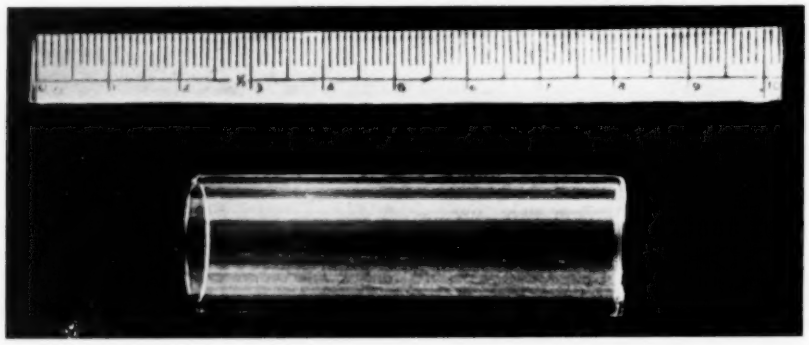

Fig. 3.—Vial for antigen dilution (exact size).

Vials.—Vials for preparing the antigen dilution similar to those recommended by Kahn are satisfactory although those of a somewhat larger size (6 cm. in length, 2 cm. in outside diameter) are preferable. (See Fig. 3.)

Antigen.—The antigen (an ether insoluble alcoholic extract of beef heart powder) and the antigen dilution are prepared as for the Kahn test. It is important that the antigen contains no precipitate at room temperature. If after cholesterolization and filtration of the antigen a precipitate forms at room temperature, this should be removed by placing the antigen on ice for an hour and then filtering it through filter paper. In the future it is proposed to place the cholesterolized antigen on ice for an hour before filtering it.

Antigen Dilution.—The antigen dilution should be made up just before pipetting the sera. Some antigen dilutions have been found to work only within fifteen minutes of their preparation. An average antigen dilution may still be used forty-five minutes after its preparation. Some antigens work best beginning about ten minutes after their preparation, others work well beginning immediately after their preparation. The action of the antigen dilution in the slide test has been found unsatisfactory at low temperatures. Accordingly it is important that the room temperature be no less

than 70° F. and that the glassware and ingredients be not cold. A blotter or piece of felt on the table top upon which the tests are set up is advisable.

Sera.—These are obtained as for the Wassermann test, care being exercised that they contain no red blood cells or foreign material. Before use, they are heated to 56° C. for one-half hour.

THE IMMEDIATE SLIDE PRECIPITATION TEST FOR SYPHILIS

Into each of the twelve rings on the slide, 0.05 c.c. to 0.06 c.c. of the undiluted serum to be tested is delivered from a pipette. The tip of the pipette is placed in the center of the ring and the serum allowed to run out. After all the sera are pipetted one drop of the antigen dilution (0.0075 to 0.0085 c.c.) is allowed to fall from the capillary pipette into the serum in each ring. After all the antigen is pipetted, the small amount in each ring is evenly distributed by stirring the mixture with a toothpick (a new toothpick with flat end a few mm. in width is used for each test). After the twelve mixtures have been made, the slide is rocked and rotated between the thumbs and index fingers of both hands for two to three minutes (depending upon the character of the antigen dilution) and read immediately. To insure even movement of the slide, both wrists and hands should participate equally. The slide should not be fixed or relatively fixed by one hand and moved by the other. Any spilling from a chamber makes the reaction therein unsatisfactory and the serum concerned should be retested. The readings are made through the microscope (16 mm. objective, 10 or 12.5 eyepiece) with the light cut down as in studying urinary sediments and recorded in terms of pluses according to the degree of clumping and the size of the clumps. Because of their importance, it is strongly recommended that all the tests be done in duplicate, using different antigens.

COMMENT

The microscopic slide precipitation test for syphilis has been further simplified and improved. As now recommended it requires no incubation, no humidor cover and may be done in a room regardless of its humidity. For the twelve tests set up at one time, a microscopic slide 2 by 3 inches holding twelve paraffin rings is used instead of three slides 1 by 3 inches, each holding four paraffin rings. The use of an antigen clear at room temperature and its more thorough immediate mixture with the patient's serum gives better results than the previous method.

CONCLUSION

The microscopic slide precipitation test for syphilis with Kahn's antigen dilution has been further simplified and improved. The immediate test, described in this paper, is as specific as the Kahn and Wassermann tests and has the advantage over those methods in that it is much simpler and requires much less apparatus and serum.

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LEUCOCYTIC INDICES OF BODY RESISTANCE WITH REPORT OF A NEW INDEX*

By Walter Clinton Jones, A.M., M.D., F.A.C.S., and Fred L. Crocker, B.S., Birmingham, Alabama

THE development of our knowledge concerning the clinical significance of total and differential leucocyte counts, especially in reference to acute infections, has crystallized very largely around five articles by Sondern[1] published in 1905 and 1906. He analyzed a very large series of cases and drew the conclusions that the resistance of the patient is measured by the total number of leucocytes, and the severity of the inflammation, by the polymor-

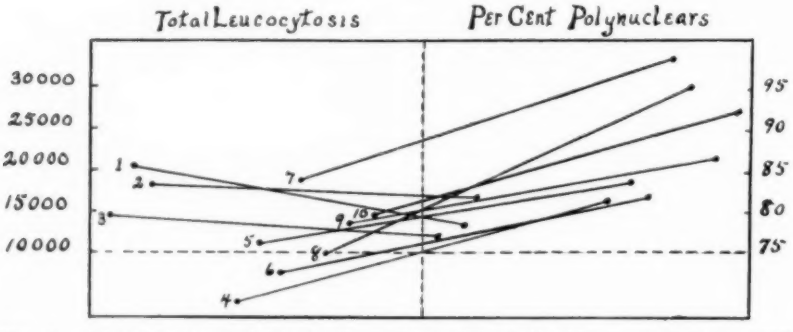

Fig. 1.—Gibson's chart of leucocytic indices. The left side is used for recording the total counts and the right side, the percentage of polymorphonuclears. The numerals in the vertical row at the left indicate the size of the total counts, while numerals similarly arranged at the right designate the various percentages of polymorphonuclears. The numerals on the two sides are arranged so that a rise of 1 per cent in the polymorphonuclears corresponds to an increase of 1000 in the total white count, starting with 75 as the highest possible normal percentage of polymorphonuclears and 10,000 as the largest possible normal number of leucocytes. The arabic numerals (1 to 10) at the left ends of the index lines indicate the various counts which were made. If the two leucocytic elements are increasing or decreasing proportionately, the lines are approximately horizontal and indicate a normal resistance (Counts 2 and 3). If the total count is higher than the percentage of polymorphonuclears, the line runs downward and indicates an especially favorable condition (Count 1). If the total count is lower than the polynuclear percentage, the line runs upward and signifies an unfavorable prognosis (Counts 4, 5, 6, 7, 8, 9, and 10).

phonuclear percentage. These probably are the two most fundamental hematologic principles on which depend the clinical interpretation of leucocytic counts.

The first attempt to correlate certain elements of leucocytic enumerations for the purpose of working out a clinical index of the patient's condition was made, as far as we can ascertain, by Gibson,[2] in 1906. He contended that the chief value of the total count and the percentage of polymorphonuclears consisted in considering them strictly together and constructed an ingenious

*From the Department of Clinical Pathology of the Employees' Hospital of the Tennessee Coal, Iron & Railroad Company, Fairfield, Alabama.

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chart for this purpose. (See Fig. 1.) When only a small number of counts are recorded, it serves its end without serious difficulties. As the counts increase in number, however, the lines become so numerous and are superimposed upon each other to such an extent that it is difficult to follow consecutively the various counts. Nevertheless, this chart has served a very

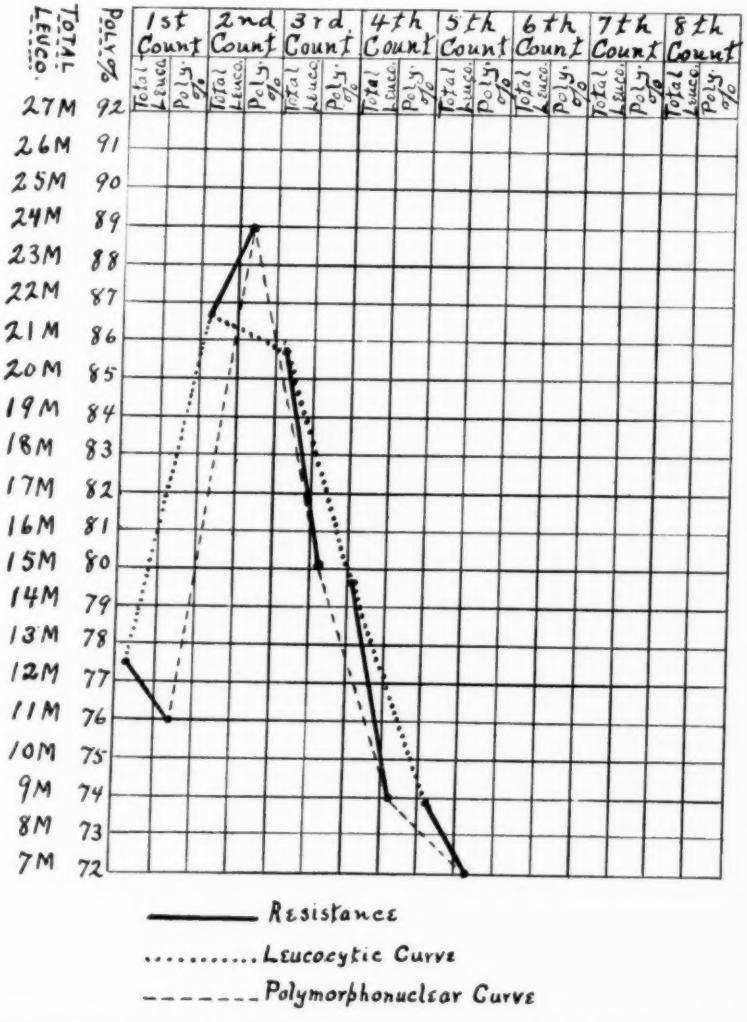

Fig. 2.—Wilson's chart of leucocytic indices. When the resistance (heavy) lines slant upward, the prognosis is good; when downward, it is bad. For further description, see text.

useful purpose; and to Gibson belongs great credit for originating such an important principle of procedure.

Wilson[3] (1908) modified Gibson's chart by making two vertical columns for each count, the left for the total count and the right for the percentage of polymorphonuclears, the same as in Gibson's chart; but Wilson made the columns narrower. (See Fig. 2.) Very few of the main index lines cross each other; hence, one can read the tables with perfect ease, no matter

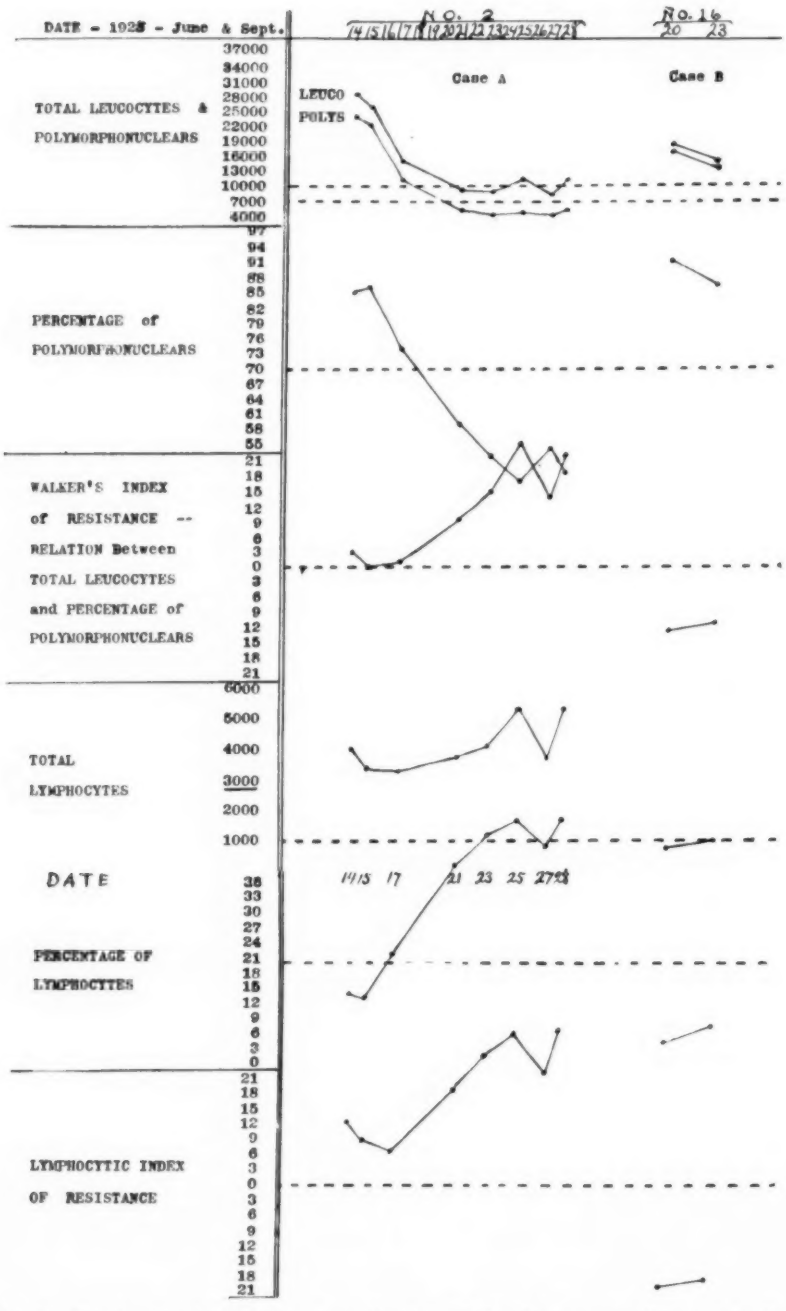

Fig. 3.—The heavy straight line near the top of the figure, extending out to the right from "10,000," indicates the highest possible normal number of total leucocytes per cubic millimeter. A similar line extending to the right from "7,000" signifies the highest possible normal number of polymorphonuclear leucocytes per cubic millimeter. The next heavy line below, extending out from "70," marks the highest possible normal percentage of polymorphonuclears. The heavy line extending to the right from "1,000," indicates the lowest possible normal number of lymphocytes per cubic millimeter; and "3,000" is underscored to signify the usual high normal number of lymphocytes. The heavy line drawn from a point between "21" and "18" represents "20," the lowest possible normal percentage of lymphocytes. The lowest heavy line extending to the right from "0" indicates the normal lymphocytic index of resistance.

how large the number of counts recorded. He added also two new features by connecting the points for the total counts with dotted lines and by joining the points marking the polymorphonuclear percentages with dash lines. Thus, four features are depicted clearly, namely, the resistance of the patient, by the direction (up and down, as in Gibson's chart) of the heavy line; the severity of the process as a whole, by the height of the heavy line above the base of the chart; the changes in the total count, by the dotted line; and the polymorphonuclear enumerations, by the dash line. Moreover, all of these features can be read consecutively with clearness and precision. Thus, Wilson's chart marks a distinct step forward.

Several years later (1919), Walker[4] originated a method of expressing in chart form the relation between the total leucocyte count and the percentage of polymorphonuclears, which he called "an index of body resistance." (See Figs. 3, 4, 5, and 6.) He takes 10,000 as the highest possible normal total count, and 70 per cent as the greatest possible normal proportion of polymorphonuclears. For each rise of 1 per cent in the latter, there should be a corresponding increase of 1000 in the polymorphonuclears above 10,000. Thus, if the polymorphonuclear percentage is 80 (ten points above 70, highest normal), the total count should be 20,000 (ten points above 10,000, highest normal) in order to make a normal, or zero, index. If the total should rise to 25,000 (the percentage remaining at 80), the index would be + 5; if to 30,000, + 10, etc. On the other hand, still assuming the polymorphonuclears to be 80 per cent, if the total count should remain at 15,000, this figure would be 5000 lower than it should be, making an index of − 5; if it were only 10,000, the index would be − 10, etc. This index is computed easily and the method of recording is graphic. (See Figs. 3, 4, 5, and 6.) Also comparison with other blood counts and with such clinical features as pulse, temperature, and respiration is greatly facilitated. We have verified Walker's index thoroughly and find it exceedingly valuable, as we have shown in a previous article.[5]

Another special blood index of resistance is that of Audain[6] who compares the given total polymorphonuclear and total lymphocyte counts each with its normal. He also introduces the new feature of using the lymphocytic index if the organs involved are rich in lymphoid tissue; and the polymorphonuclear index, if they have little or none of this tissue. He does not present any records in chart form, and his indices have not been confirmed, so far as we know.

One more feature of leucocytic enumerations considered as prognostic indices should be considered in this connection; namely, the number of

Vertical distances are adjusted so that the interval allowed for a change of 1 per cent in the percentages of polymorphonuclears and of lymphocytes equals the space allowed for a change of 1,000 in the total leucocyte count. For computations of indices, etc., see text.

Case A: Acute, purulent, gangrenous appendicitis. Patient was ten years old. The very low percentages of polymorphonuclears (with a total count not correspondingly reduced) allowed the indices to rise unusually high during the latter part of convalescence. Note the fall of the indices June 27, when the patient walked for the first time. It is a common occurrence for the indices to be depressed temporarily when a patient sits up in bed or gets into a chair or walks, for the first time.

Case B: Acute, purulent, gangrenous appendicitis. Age of patient, fifty years. The two counts charted here were taken ten and seven days before the patient died. Note the low readings of the indices, especially the lymphocytic index.

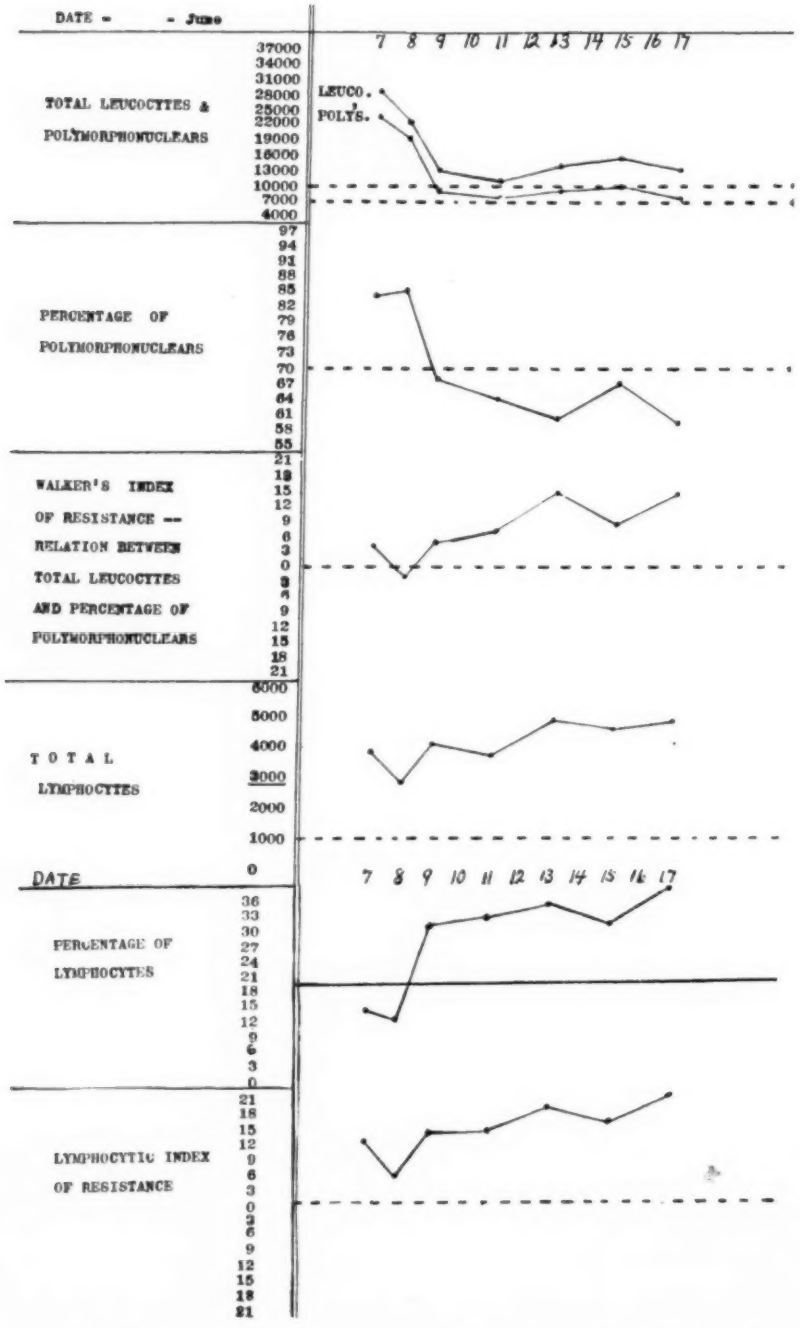

Fig. 4.—Eclampsia. Age of patient, thirty years. Convulsions occurred June 6, and a cesarean section was done the same day. The fall of the indices June 8 might be attributable to the mental and nervous depression caused by the death of the baby. From this point, recovery was uneventful, save more or less rise in temperature from the thirteenth to the eighteenth day, the highest point being 101°. During the first half of this period, the indices fell somewhat. For general description of chart, see Fig. 3. For computation of indices, tion of indices, etc., see text.

lymphocytes per cubic millimeter considered alone, independent of any other details of a blood count. While this point is mentioned by many writers, Holmes[7] (1905) considers it more thoroughly than any one else. He emphasizes the importance of studying the small lymphocytes, although most of the other writers include all of the lymphocytes, both large and small; while Gilbert[8] grouped together for the purpose of diagnosis all of the lymphocytes, the large mononuclears, and the transitionals also. Holmes also stresses the value of knowing the number per cubic millimeter rather than the percentage. He alone points out the important fact that the percentage may deviate greatly while at the same time the total number varies within narrow limits. The normal number of small lymphocytes per cubic millimeter he states to be from 1100 to 3000.

In a very large variety of acute and chronic conditions and diseases, these various mononuclear elements were found by Holmes and many others to increase almost without exception when the patient was progressing favorably, and to decrease when the outlook was bad. In tuberculosis, Gilbert,[8] Ullom and Craig,[9] Morgan,[10] and Murphy and Ellis[11] all hold that an increase in the lymphocytes (entire group) corresponds with an increase in the resistance of the patient and that a low count almost always is accompanied or followed by poor clinical progress. Hess[12] found that in children a drop in the neutrophil count with an increase in the lymphocytes indicates a good prognosis. Murphy and Sturm[13] seem to have shown by very conclusive experimental work with mice that one of the constant factors in immunity to cancer is the presence of lymphocytes, especially locally at the site of inoculation. Gilbert[8] has demonstrated that higher altitudes and Taylor[14] that exposure to sunlight usually produce an increase in the lymphocytes of the blood, while Sauer[15] discovered a lymphocytosis in functional nervous diseases, especially hysteria and neurasthenia.

Of the various indices described above, we favor Wilson's and especially Walker's.

PERSONAL INVESTIGATIONS

1. *The Lymphocytic Index.*—In studying numerous charts like those shown in Figs. 3 to 6, the senior author noted that a significant curve could be constructed by using only the total white count and the percentage of lymphocytes. (See Figs. 3, 4, 5, and 6.) This curve may be designated the "lymphocytic index," while Walker's might be called the polymorphonuclear index. Our index is computed as follows: After an extensive study of the subject, we came to the conclusion that the smallest possible normal percentage of lymphocytes is about 20. Using this figure as the lowest limit, for every drop of 1 per cent of the lymphocytes below this point there should be a rise of 1000 in the total count above 10,000, the highest possible normal for the latter. For example, if the lymphocytic percentage should fall to 14 (6 below 20), the total leucocytes should rise to 16,000 (6000 above 10,000). In this case the index would be zero, or normal. If the total count goes higher than this, the index is plus; if it falls lower, the index is minus. Thus, in the above illustration, if the total count had risen to 23,000 (the percentage of

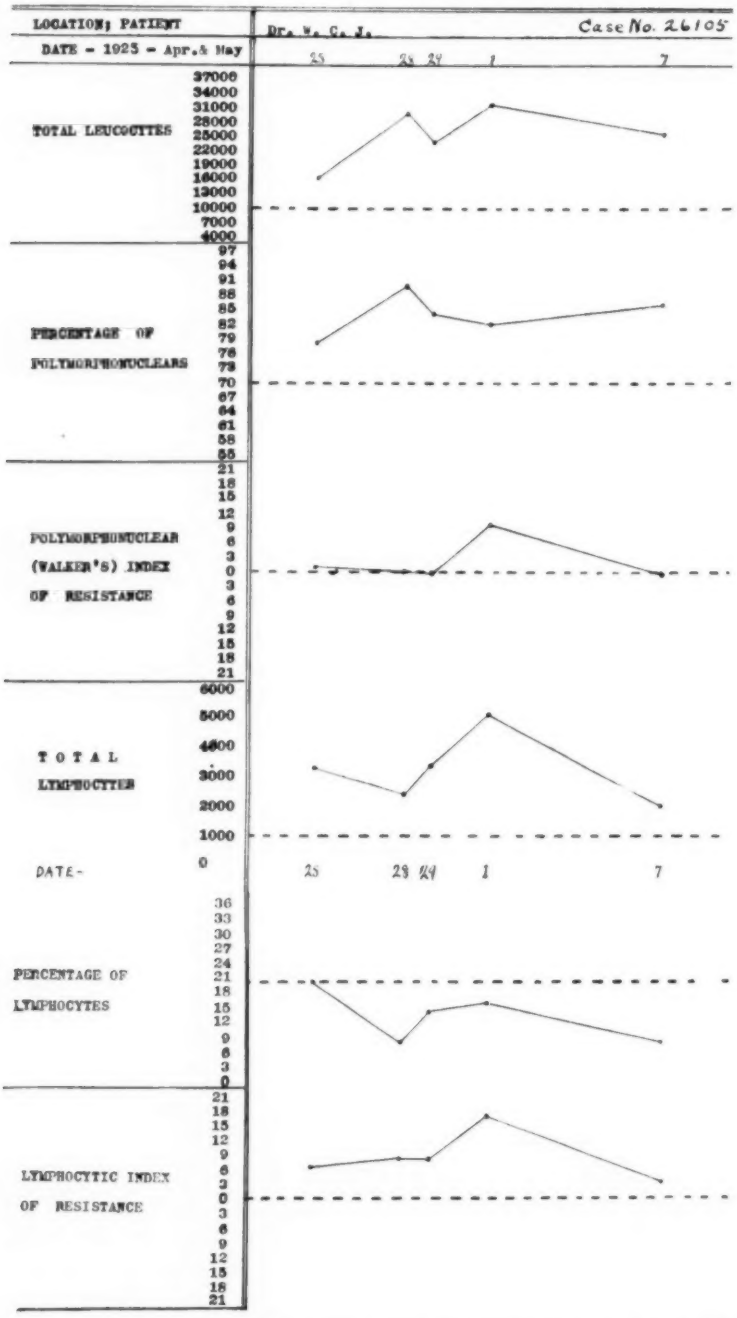

Fig. 5.—Carbuncle of neck. The patient, who was fifty-one years of age, grew worse from April 25 to 28, when an excision was done. Note the rise of the indices soon after this operation. They correspond very closely to excellent clinical improvement. From two to six per cent of eosinophiles were present constantly in this case. This is a good omen. For general description of chart, see Fig. 3. For computation of indices, etc., see text.

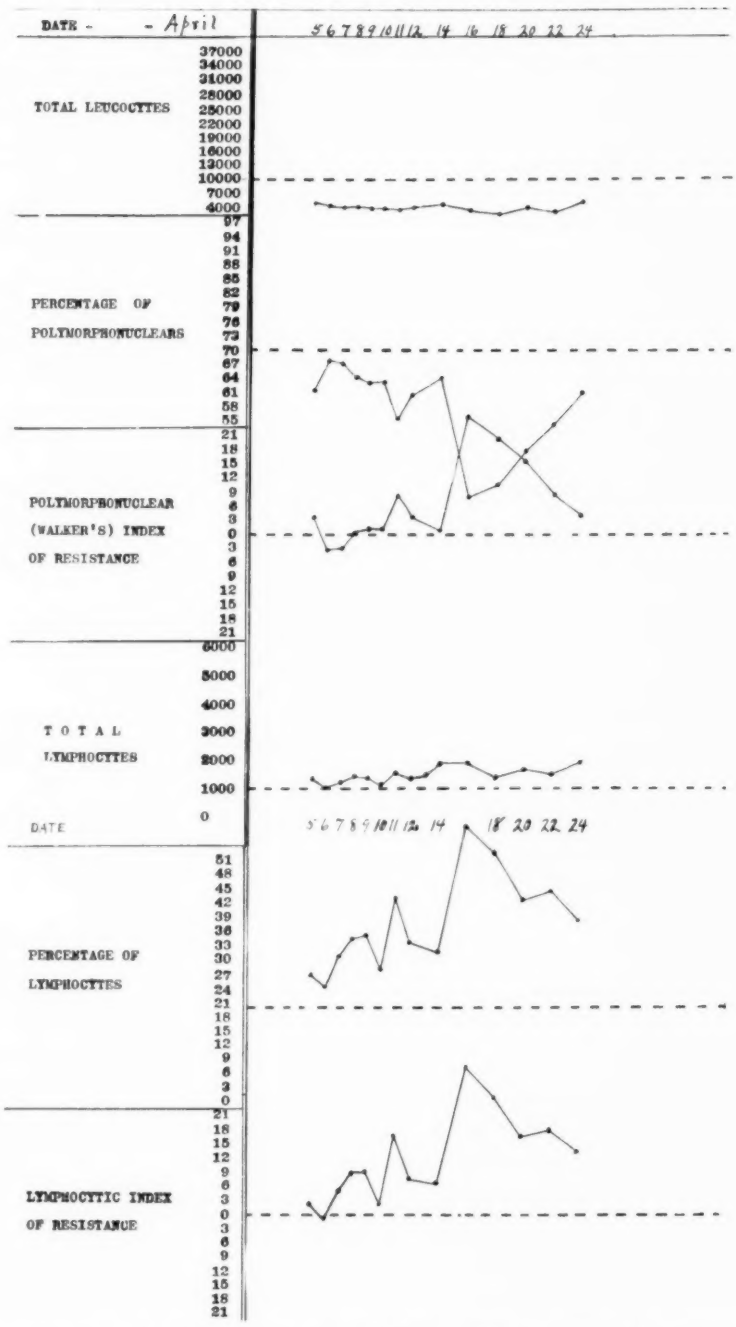

Fig. 6.—A case of typhoid fever which ran an ordinary course with recovery. Note that the total leucocytes and the total lymphocytes remain almost constant. This fact explains the almost mechanically accurate interdigitation of polymorphonuclears with the polymorphonuclear and lymphocytic indices and with the percentage of lymphocytes. This chart is a perfectly typical one for typhoid fever. For general description, see Fig. 3. For computation of indices, etc., see text.

lymphocytes still remaining at 14), the index would have been + 7 (23,000 minus 16,000, omitting the three final ciphers). Similarly, if the total count had failed to go higher than 9000, the index would have been − 7.

We find that the lymphocytic index averages about six points higher than the polymorphonuclear one. Why this is true we have not been able to explain adequately. It seemed at first that this difference might be due to the exclusion in our calculations of the endothelial leucocytes, eosinophiles, etc.; but on adding together the percentages of polymorphonuclears and lymphocytes and subtracting this sum from 100, the results average about 4, which is only two-thirds of 6, the figure representing the disparity in height of our index above Walker's. The two indices closely parallel each other, as one can observe by referring to Figs. 3 to 6. Also, they are followed very closely by the curve of the total lymphocytes, as we have shown in a former article.[5]

As to the prognostic value of the lymphocytic index, in many instances it seems to be more accurate than Walker's; and in some cases the latter is better. On the whole, we believe the best results are obtained by using an average of both.

2. *When to Use Leucocytic Indices.*—The blood findings discussed in this paper are most significant in acute infections when the percentage of polymorphonuclears is 70 or above and that of the lymphocytes, 20 or lower. However, the indices are of definite value in typhoid, tuberculosis, etc.; this is especially true of the curve of the total lymphocytes, and the lymphocytic index.

3. *Extreme Leucocytic Findings.*—Percentages of polymorphonuclears above 90 should be accompanied by total counts high enough to make a plus index of 15 or more, in order to have a favorable prognosis. For example, in a recent case of pneumonia, the polymorphonuclears rose to 96 per cent, but the total count was 79,000. These figures give a lymphocytic index of 50 and a polymorphonuclear index of 40. This patient recovered promptly. Patients with percentages of polymorphonuclears as high as this usually die, the total white count generally ranging below 50,000.

4. *Rules for Normals in Children.*—During the first year, the normal percentage of lymphocytes is about 55 and of the polymorphonuclears, about 30. The former figure gradually decreases and the latter increases until about the eighth year when they become almost exactly reversed. The senior author has formulated the following rules for ages up to the eighth year. For polymorphonuclears: After the first year, multiply the number of the year by 3 and add this product to 30. Thus, the percentage of polymorphonuclears at five years of age should be about $(5 \times 3) + 30$, or 45. For lymphocytes: After the first year, multiply the year by 3 and subtract this product from 55. For example, the number of lymphocytes at the age of 6 should be about $55 - (6 \times 3)$, or 37. After the eighth year, the percentages are practically the same as in adults.

5. *Eosinophiles.*—These leucocytes usually disappear during an acute infection. However, their persistence or their reappearance is practically always of favorable significance.

6. *Correlation of Blood Findings with Clinical Symptoms.*—It is frequently necessary to emphasize this feature. Mere following of blood indices will lead to many errors. For example, both a normal and a seriously sick person may have a zero (normal) index. It is self-evident that we cannot have the same attitude toward both of them.

SUMMARY

1. The fact that the percentage of polymorphonuclears is a measure of the severity of an acute infection and that the total white count indicates the degree of the resistance of the patient constitute the two most fundamental principles underlying the application of leucocytic counts to clinical cases. These principles were established about twenty years ago by Sondern; and on the basis of these discoveries, Gibson, and Wilson worked out very useful clinical index charts.

2. The total number of lymphocytes per cubic millimeter (not percentage) constitutes a valuable clinical index. Holmes has had a very large part in establishing this principle. This index is useful in both acute and chronic infections but particularly in the latter, especially tuberculosis.

3. A lymphocytic index of resistance has been worked out by the senior author, using the total white count and the percentage of lymphocytes. This index parallels Walker's very closely but averages about 6 points higher.

4. Polynuclear indices are useful chiefly in acute infections. Lymphocytic indices are of value in both acute and chronic inflammations.

5. When the percentage of polymorphonuclears rises much above 90, the total white count should reach 50,000 or more in order to justify a favorable prognosis.

6. In children during the first year, the normal percentage of polymorphonuclears is about 30 and of the lymphocytes, about 55. These figures gradually interchange until they become reversed during the eighth year.

7. The continued presence of eosinophiles or their reappearance during the course of an acute infection is a favorable sign.

8. One should not fail to correlate carefully laboratory blood findings with the clinical symptoms.

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THE DIFFICULTIES AND VALUE OF FROZEN SECTION METHODS*

By L. A. Turley, A.M., Ph.D., Norman, Okla.

AT EVERY meeting of this Society in the last several years, the question as to the value of frozen sections in tissue diagnosis has come up and the society has been pretty much divided into two camps, those in favor and those opposed to frozen sections. At the meeting last year in Philadelphia, if you will remember, I promised to present at this meeting a discussion of frozen section methods and it is to keep this promise that I am presenting this paper.

The purpose of any histo-technical method, is to enable us to get a clear conception of the structure of cells and tissues and their relation and arrangement in organs. Therefore, any method must be judged on the basis of the exactness of the picture which it affords. Every one who is familiar with the usual embedding methods or any one who has studied histology and organology from sections made by these methods, has been more or less dissatisfied for a number of reasons. First, it is a long drawn out process, in some cases requiring weeks of time and in many instances requiring infinite care and patience with the individual steps; and second, any of these methods yield results which, beyond any doubt, give a distorted picture of what the tissues actually look like in life. These difficulties arise, because in the first place, the tissues must be subjected to the action of some agent known as a fixative which preserves the tissues in the exact condition they were in when the specimen was taken. These fixatives coagulate or precipitate certain of the protoplasmic constituents and also cause a shrinkage of certain tissue cells, and elements, more notably smooth muscle and collagen fibrils, and if applied in too great concentration will cause the shrinkage of

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cells, for example, certain types of epithelium, hence the distortion is apt to be very great. Second, since all embedding substances are insoluble in water, it is necessary to dehydrate the tissues before they can be embedded and cut, which again gives rise to possibility of distortion unless great care is exercised to not hasten the dehydration process. Third, after tissues are cut it is necessary to rehydrate them in order to stain them, and again dehydrate them for mounting, so that it is perfectly evident that this method must be carried out with utmost care in order to get sections that are at all satisfactory and even then we must recognize that the tissues are somewhat distorted. These disadvantages are offset to a certain extent by the accurate preservation of tissue structure and even of intracellular structure, both of which are of extreme importance in obtaining an accurate knowledge of tissue structure. In fact so important are these structures to an exact knowledge of tissues, that their preservation outweighs, practically, all objections to the embedding method although it is necessary at all times to remember that we are looking at distorted specimens. Another point in favor of this method is the acuteness of the chromatophilia of the cells and cell elements which are highly differentiated, without a view of which the fine points of tissue structure and cellular identification cannot be determined. But it is quite obvious that such a method whatever its merits, because of the time involved, never could be used if a rapid diagnosis of tissue were necessary.

With the discovery and development of the frozen section method, histologists and histology technicians at once expected emancipation from the difficulties attendant upon the embedding method. However, they are doomed to disappointment, for many reasons. In the first place, in the process of freezing and thawing, there is bound to be more or less loss of tissue continuity and even the loss of a great many, if not of all parenchymatous cells, in sections of organs and from lesions where the stroma is scanty; and second, in the handling of these unsupported delicate sections, tissue relations are often distorted, for example, too strong a pull with a camel's hair brush or a glass rod on one side of a section will make the tissues appear edematous. It was also found that unfixed tissues do not stain as readily nor as sharply as do fixed tissues, so that fine points of intracellular and intercellular structure cannot be made out. However, there is an increasing demand on the part of clinicians for an exact knowledge of the nature of lesions, with which they are dealing, especially in surgical procedure. At the Mayo Clinic, surgical specimens are examined and the surgeon furnished with a diagnosis while the patient is under ether. Dr. Bloodgood has the staining and examination process performed in the operating room so that he not only is furnished with the diagnosis during the operation, but is enabled, if he so desires, to step to the microscope and see the character of the lesion himself.

A careful and conscientious surgeon wants to, and all surgeons should, as nearly as possible, know the nature of the lesion with which he is dealing while the patient is under the influence of ether and the wound open so that he will know how to proceed. An examination of the records of any hospital clinic or private practice will show that mistakes in diagnosis are made

without tissue examination and procedures carried out on the basis of those diagnoses that not only fail to benefit the patient, but often injure him, sometimes hastening his demise. I need not cite these examples from my own experience. Look at the records of your own clinics and you will find them. These mistakes are often made when every known diagnostic method except tissue examination is employed. This is no criticism of surgeons, clinicians, nor clinical pathologists. These mistakes are due to the finiteness of human knowledge and the lack of employing the right diagnostic aid. If it were not for the first of these factors we would not be here, for clinical pathology would not be a branch of medicine. No surgeon can tell the exact nature of a lesion in every case without tissue examination any more than an internist can tell in every case the lesions with which he is dealing without the aid of laboratory procedure. The tissue examination is to the surgeon what the Wassermann, Widal, urinalysis, blood chemistry, basal metabolism, etc., is to the internist. Therefore, it is imperative that some method of rapid tissue diagnosis be devised that is much shorter than the embedding method, and it falls upon us as clinical pathologists, in the fulfillment of our calling as the makers and interpreters of exact diagnosis and in justice to the patient and clinician to devise and employ some method of rapid tissue diagnosis that is suitable for diagnoses while the patient is under the ether. For this reason a discussion of methods of rapid tissue diagnosis is not only timely and desirable, but becomes as much our duty, and should be as much our interest, as the discussion of any laboratory method, bacteriologic, serologic or chemical. It does not get us anywhere to dwell on the difficulties of rapid tissue diagnosis, and recount the failures or possible inaccuracies of the method unless we at the same time attempt to devise means for overcoming those difficulties and minimizing the number of possible inaccuracies. What clinical pathologic method is 100 per cent perfect? Over one-half of one of our programs was taken up with discussions of the Wassermann reaction and suggestions as to how it could be made more exact and reliable and yet it is perhaps the most reliable of all laboratory procedures. In the few minutes of time that remains to me, therefore, I wish to bring out some of the difficulties of frozen section methods and suggest ways in which these difficulties can best be combated or minimized and also the methods for obtaining the best results.

In the first place, in freezing blocks of tissue, care must be taken not to freeze the tissues too hard or cut them while they are frozen too hard because during the thawing out process the rapid change in temperature will set up currents which will tear to pieces tissue composed of unsupported cells. I am sure that all who have tried the frozen method have seen sections, when placed in water from the knife, fly to pieces almost as if they had exploded. Therefore, the temperature range must not be too great and the thawing process should not proceed too rapidly. This can be brought about by not freezing the tissues harder than is necessary for actual cutting, and the slow thawing can be accomplished by either allowing the section to thaw on the knife, preferably, in a drop of liquid that can be carried on the

knife, or as Terry recommends, on the finger, and if this detail is observed one will be surprised how perfect a section, even of very cellular tissue, may be obtained by the frozen method. In the second place, further tissue distortion can be obviated by care in handling. If the sections are to be mounted on slides by the use of egg albumen or otherwise, they should be floated and not dragged into position. It is remarkable what a perfect tissue arrangement can be preserved by this procedure.

We now come to the staining. It is perfectly obvious that the staining and clearing process must be done quickly, otherwise much time is lost. But it is just as obvious that any staining method used must give a cellular differentiation clear enough that the tissues may be quickly recognized and their condition determined, and, further it is necessary that the preparation be of a permanent character so that it may be mounted and preserved, for as has been pointed out before, the slide from which a diagnosis is made is as much a part of the record in a case as the diagnosis itself. For general all-round purposes, no method of staining has yet been devised that surpasses or that is equal to the hematoxylin-eosin method. But this is rather an elaborate method requiring a good many steps so that the actual staining by this method requires ordinarily two or three minutes as a minimum, which of course seems a long time to a surgeon standing beside a patient under ether, and waiting to know how to proceed. Various attempts at developing shorter methods by use of other methods and especially using polychrome stains have been tried and although some of them are very rapid they lose so much of detailed differentiation that a diagnosis from such a stained specimen is much more difficult to make, and time gained in the staining is lost in the additional time required in the examination of the stained specimen. Terry reports that by his polychrome method, a section may be stained in ten seconds, and McCarty, using this method has stated that he has made a diagnosis in fifty-two seconds from the knife, but in Terry's own words it is practically necessary for the pathologist to learn a new cytologic interpretation because of the lack of sharp differentiation, and also that these sections fade in a few hours and must be restained by another method if a permanent record is desired, which, as pointed out above is all-important, which means additional work that is unnecessary if speed and permanence can be obtained by the one process.

Realizing the necessities, the criticisms and aims of frozen section work, the author conducted a series of experiments trying, if possible, to obtain a section under the microscope with a good differentiation in a very short time. Of course the interpretation of a slide picture depends upon the skill of the pathologist. The time the surgeon has to wait for a diagnosis depends not on the staining method alone, but also on the skill of the pathologist in interpreting the slide picture. The staining method is employed merely as an aid to the pathologist. Therefore, it should be as rapid as possible to give him a larger percentage of a given time to examine the specimen. Also, since it is merely an aid to give him a picture, it must be as clear as possible, in fact clearness of the picture is the more important of the two considerations.

And in the end more time will be saved if a clearer picture is obtained by a little longer process so that the examination may be shortened, than by a very rapid process which gives a hazy picture which requires more time to study. So the aim of rapid procedures should be the shortest possible process that gives clear pictures. This was the aim of the author in the following procedures.

Starting with the fact that fixed tissues give good clear pictures by the frozen method, an attempt was made to introduce a fixing process into the frozen method. It was found that if sections, after being mounted on a slide and before the staining was begun, were flooded with 4 per cent formaldehyde for a few seconds, the subsequent stain was much faster, clearer and more definite. Therefore experiments were tried floating the sections from the knife in formaldehyde solutions of various strengths. It was found that formaldehyde in concentration of 4 per cent could be used in place of water and this accomplished several things: First, a fixation which preserved tissue structures in as near as possible the natural condition in which they were in the body; second, by the coagulation of intercellular and intracellular structures the tissues do not tend to disintegrate after being cut, and resist the deleterious effects of handling to a very great degree; and third, increased the speed and sharpness of the stain. Emboldened by the rigidity and toughness of the sections thus treated, experiments were carried on to see if the mounting process could not be delayed until after the staining and clearing, thereby improving the stain both as to shortness of time and greater exactness because the time necessary to fix the section to the slide would be saved and the cells would stain from both sides of the section instead of from one, as is in the case of the mounted section. To our great satisfaction it was found that this could be done, so here again considerable time is saved and mounting media could be dispensed with. The method is as follows. Sections are thawed on the knife by cutting them into the small amount of fluid that will cling to the upper side of the knife previously immersed in the floating fluid. The under side of the knife must be wiped dry. Sections are floated in 4 per cent formaldehyde. A glass rod is employed for handling the sections. This glass rod is drawn into a narrow tip bent at a slight angle. The section is lifted into the hematoxylin solution. In from one to three seconds it is lifted into tap water and dipped up and down a couple of times to wash off the hematoxylin. It is then dipped into 70 per cent acidulated alcohol for differentiation, back into tap water, next ammonia water, tap water, then eosin for two or three seconds, and into 95 per cent alcohol for a second, then into beechwood creosote. If the section is plunged perpendicularly into the creosote it will unfold like the opening of a book and will straighten out, it is then slid onto a slide and can be examined in the creosote with or without a cover glass. If it is desired to preserve the section, the creosote is blotted off and balsam and cover glass are put on. This method gives a clear sharp stain and a permanent mount, as permanent as any hematoxylin-eosin stained specimen by the long embedding process. I realize that the first criticism of this method that will be made, is that there are an enormous number of steps in the procedure, but

you will be surprised at the short time required by each step, and so far as the time the whole process requires, in the hands of Mr. McHale, an untrained student, I was able to have good clear sections, under the microscope in thirty-five seconds, which, considering the excellence of the product and its permanence, is a short enough time for anyone. Also, the excellence of the product justifies the few seconds more time over the more speedy polychrome methods now in use.

Having a staining method that is accurate and rapid enough for practical purposes, there are some other important factors that must be considered if rapid tissue diagnosis is to be as serviceable as it should be. One of these is the selection of the block of tissue to be sectioned. This is in most cases up to the surgeon whether the tissue is sent to the laboratory before the operation or during the operation. Most imperfect diagnoses that are charged to frozen or rapid tissue diagnosis are due to the failure of the one taking the specimen to get a characteristic block of tissue. All that can be expected of the tissue pathologist is that he make an exact diagnosis and interpretation of the specimen furnished him. So if the tissue diagnosis proves to be wrong the first question to raise is not the pathologist's honesty or ability, but the selection of the tissue furnished him. I have, on several occasions, asked the surgeon for a second piece of tissue even when the patient was under ether and have always justified this delay by a more accurate diagnosis.

In making a tissue report from a small piece furnished for rapid diagnosis, one should always say, "Sections from *this specimen* show such and such conditions." Such a statement is the truth, it protects the pathologist from criticism, and is more satisfactory to everyone concerned, because it is all the pathologist can say and it at once notifies the surgeon that if the diagnosis was not what he expected he has the means of getting more information which is, furnishing another block of tissue from another part of the lesion.

Another important factor to be considered is the pathologist himself. Much of the criticism of frozen section diagnosis from the clinicians is because those without proper training are attempting tissue diagnosis. And much of the reluctance of clinical pathologists to do rapid tissue diagnosis is because they realize their inability to diagnose tissue changes. The recognition of tissues in abnormal conditions and to properly interpret such conditions is the most difficult of all clinical pathologic procedures. It is easy to maintain a constant temperature in a bath, to add so many cubic centimeters of this or that solution to another and watch for a precipitation or change of color or reduction of some chemical substance. It is easy to count blood cells, to recognize bacteria, etc., but to tell when hyperplasia becomes neoplasia, to recognize atypical pancreatic cells in the lungs, or atypical mammary cells in the brain is quite a different story. A tissue pathologist must be a cytologist. He must be familiar with the normal appearance of cells in the different phases of their functional life and the history of their development from the undifferentiated cells of the germ layers. He must be familiar with the differences in appearance of cells in inflammations, degen-

erations, regenerations and neoplasias. No one should attempt tissue diagnosis who does not have a thorough knowledge of histology and embryology, followed by long, extensive and careful study of histopathology.

SUMMARY

1. Frozen sections or equally rapid tissue diagnosis is as important as any clinical laboratory procedure, and it is as much up to clinical pathologists to prepare themselves for this service as for any other clinical laboratory procedure if they are entitled to the full respect and place in medicine they should have and take.

2. Good, clear, permanent mounts with good differentiation, sufficient for all clinical diagnoses, can be made in one minute or less, which is short enough time for all practical purposes.

3. We should insist on specimens from such parts of a lesion as will reveal its true character, and in all cases to state that the specimen furnished revealed the facts which led to the diagnosis made, and that the diagnosis is a diagnosis of that specimen only.

4. Tissue diagnosis is the most difficult of all laboratory procedures so should be attempted only by those specially trained.

DISCUSSION

Dr. Michael G. Wohl.—Tissue diagnosis by the frozen method is one of the links that still binds the clinician to the clinical pathologist. It is a branch of clinical pathology the execution of which cannot be delegated to the lay technician or the Board of Health. Tissue interpretation requires a medical mind and clinical judgment that can only be acquired through years of experience. We should therefore endeavor in every case to discuss the clinical side with the surgeon. The clinical pathologist should be just as much interested in the clinical symptoms of the patient as in the laboratory findings. I feel that it is a good rule to follow to be present in the operating room and see the tissue the surgeon excises. When the tissue is cut into one often may be able to tell from the naked-eye appearance as to whether or not the tissue is malignant. The clinical pathologist is able to point out to the surgeon the appearance of malignant tissue, and the surgeon will soon become familiar with the appearance of pathologic tissue when he sees it. You thus make a friend of the surgeon. The secret of obtaining good frozen sections is to do them routinely, often enough. We have obtained sections that were thin enough for microphotographs. It seems to me the difficulty lies, not in the preparation of the tissue so much as in the time allowed for the study of a frozen section. When the surgeon desires an immediate diagnosis, I would rather trust an opinion based upon a careful clinical history and the gross appearance of the tissue than a hasty examination of a frozen section.

Dr. F. W. Hartman.—It is a good thing for us to consider the whole question of frozen section diagnosis and to get the various ideas regarding the Terry stain. In our own laboratories our results have been variable. At one time a nuclear stain is obtained and at another time no differentiation is brought out even when using the same batch of stain. It has seemed that with the older method of polychroming more consistent results were obtained. Dr. Turley's method for quick sections is interesting and well worth a trial. However, it has been our experience that creosote often distorts fresh tissue and the section is often wrinkled. If so much time has to be consumed we would prefer the fixed frozen section stained with hematoxylin and eosin. We cannot agree with Dr. Wohl's remarks regarding the pathologist making a diagnosis from the gross tissue alone. If the surgeon is worthy of the name he can make his own gross diagnosis and he wants confirmation of this diagnosis from the pathologist's examination of sections. It should never be necessary to review the history after the patient is on the operating table. This should be done before the operation is started so that the pathologist will be in position to make

the histologic examination at once. In our institution we make it a rule that the frozen section diagnosis shall be tentative only and that a change may be made in this diagnosis if the permanent sections warrant it.

Dr. Philip Hillkowitz.—This question of frozen sections is of great importance to the surgeon. There is no field wherein the clinical pathologist is of greater value to the clinician. There is where the surgeon feels a dependence on the clinical pathologist; the life of the patient and the extent of the operation depend on his dictum. I am very glad that Dr. Turley has brought this matter up. It is not so much a question of the particular technic employed as it is whether the tissue is benign or malignant. I was rather shocked by Dr. Wohl's remarks. For instance, in a case of suspected malignancy of the breast where the clinician calls on the clinical pathologist for a diagnosis, it is of great importance to the surgeon to know what procedure to follow. If it is a case of adenofibroma it would be a crime if the breast were removed; the tumor could be shelled out and the breast remain. On the other hand, if it is carcinoma a radical operation with excision of the axillary lymph nodes would be done. At the same time I fully agree that in the great majority of cases the clinical pathologist is in a position to make a diagnosis with the naked eye. While the surgeon should be able to do it we know from experience he has not skilled himself to that extent. Very frequently for a skilled pathologist, it is simply going through the motion to have a frozen section made when he can tell by the naked eye but as a rule it has a good effect to demonstrate the microscopic section to the surgeon and confirm the macroscopic diagnosis.

Dr. Herman Spitz.—It seems to me that we are missing the most important point in regard to frozen sections. You will recall that during the first year of our existence as a Society, our president, Dr. Hillkowitz, carried on quite a protracted discussion with the American Medical Association in regard to advertising. As a result of that discussion, the Advertising Committee of the American Medical Association classified us as "manipulators of test tubes and other inanimate objects." Now it seems to me that here is a splendid opportunity to refute that classification. We are doctors, as much so as any other members of the American Medical Association. We are called into consultation by the surgeon when we are asked to do a frozen section. The surgeon waits with bated breath until we give him our report. If we tell him to cut wide, he does so. If we tell him he has done enough and that no further surgery is required, he is satisfied. Now, if this is dealing with "test tubes and inanimate substances" somebody should rewrite the dictionary. In the case of a young girl with a mass in the breast, it is up to the pathologist doing the frozen section to say whether that girl should keep her breast or lose it and be disfigured for life. The same argument is true in many other instances. The method of doing a frozen section, the technic employed, the stains used, etc., are matters of personal preference. The real value in this paper is the fact that it tells the Advertising Committee of the American Medical Association that we are real doctors, dealing with life, just as all other physicians deal with life, and that we do something besides "manipulate test tubes and other inanimate substances."

Dr. E. F. Cooke.—I don't believe it makes so much difference which technic is used. I have seen beautiful sections by various technics, and even by freehand cutting. What I object to, is the hurried diagnosis. It is not the obvious case that is important. In such a case the surgeon himself is in no doubt, and does not need our aid to make his decision, but it is the borderline case, the one that we even find difficulty in diagnosing after we have our sections cut. I feel sure that all here who do tissue work, have right now, specimens lying on the table that we are hesitating to express an opinion upon, and they may have been lying on the table for a week or ten days. It has been argued that one can make a hurried frozen section diagnosis, and then confirm or modify the same after a more deliberate technic and study, but it is quite true of human nature that we hate to take up a task that we thought completed, it is quite a bore to take up a tissue again and go on further with it. And it is quite embarrassing to have to change or modify a diagnosis. About seventeen years ago I could make a prompt diagnosis of any tissue. I cannot do that now, maybe it is because I am getting old and cannot see. Some time ago I had the pleasure of seeing the tissues from the Bone Sarcoma Library of

Atlanta, Ga. and it was interesting to see the different diagnoses that had been rendered by different pathologists on the same tissues. This was not only interesting but consoling to a man like myself. I object to the surgeon who wants to hurry you to an express train diagnosis, when he has probably been studying the case for weeks before he dared operate.

Dr. L. A. Turley (closing).—Dr. Wohl makes two very important points, one of which is that tissue examinations cannot be delegated to lay technicians or Boards of Health. As was pointed out in my paper, tissue diagnosis requires the most careful expert preparation of any branch of clinical laboratory procedure for reasons above pointed out. The other one with regard to Boards of Health, tissue should never be sent to Boards of Health for the reason that they can never be Public Health matters and further no Board of Health staff should or can afford one capable of doing tissue diagnoses. Another point made by Dr. Wohl is that frozen sections should be made often enough, whether requested or not by the surgeon, to train the technician in the cutting and staining, and the pathologist himself in interpreting frozen sections.

On some other points of Dr. Wohl's discussion, I must heartily disagree. That is, the making of diagnoses from the appearances of gross specimens without microscopic examination. If time permitted, I could cite cases from the best laboratories in the country where the opinion was arrived at by the most expert clinicians and pathologists that the gross diagnosis was proved erroneous by tissue examinations. I am not pessimistic when I say that I doubt whether any pathologist, regardless of his innate ability, his training and experience, however prolonged, is capable of making gross diagnoses that are exact and reliable in a sufficient number of cases to be absolved from a necessity of checking up such diagnoses with tissue examinations. Such a statement is based on the point made above which is the finiteness of human knowledge which will prevent a full appreciation and realization of the varied appearances and conditions met with. Even if one sees the tissue in situ, the distortion due to light, mechanical manipulation, presence of hemostats and other instruments, discoloration by hemorrhage, and other factors confuse the picture so that it is more difficult to make a gross diagnosis in situ than after the tissues are removed. Even under these circumstances, gross diagnoses cannot be made that are sufficiently reliable to dispense with microscopic examinations. It often happens that malignancies are found on microscopic examination that are not suspected on clinical examination nor gross inspection. Further, as Dr. Cooke stated, it is the borderline case and the unsuspected case where the tissue pathologist serves his most useful purpose. If the case is perfectly obvious, the services of the tissue pathologist are not necessary. There are many mounds in cemeteries that are monuments to the conceit of clinician's and pathologist's ability to make diagnoses from clinical facts and gross inspections.

The point brought out by Dr. Hartman in his discussion that creosote often wrinkles tissues is true but is no criticism against the frozen section methods. If you will take a binocular microscope and examine sections made by any technic however prolonged and careful, you will find wrinkles that were not suspected and which often distort the picture. However, I think Dr. Hartman will find that if sections are fixed as outlined in the paper and a little more time spent in the dehydration process, he will have less trouble with the creosote wrinkling.

The point brought out by Dr. Cooke with regard to the time often necessary to make a diagnosis is also no criticism of frozen section methods. I have personally known some of the ablest tissue pathologists now living to spend from three weeks to six months studying specimens from a case before they are willing to make a diagnosis even when they had employed not only the long embedding method but also differential stains. The frozen section method is intended to give the greatest amount of information in the shortest space of time and, as stated above, has never, and probably never will supplant the longer embedding methods for exact diagnoses. When this is fully understood, I think no one should feel abashed or ashamed of having to revise his diagnoses any more than a clinician should in revising his diagnoses on the basis of more complete and more exact information.

In closing, I will say that the method presented is neither the only one nor perhaps the best one. All that is claimed is that by employing this method one can get a very exact picture and a permanent mount, both of which are of supreme importance in tissue diagnoses.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

Mellon, R. R.: The Infective and Taxonomic Significance of a Newly Described Ascospore Stage for the Fungi of Blastomycosis. Jour. Bacteriol., April, 1926, xi, No. 4, p. 229.

In a paper illustrated by 27 microphotographs Mellon describes observations furnishing evidence for the formation of four-celled asci with Types I and II of the parasites of blastomycosis as described by Ricketts, this perfect stage representing a form of endosporulation not previously described.

This phase of development seems to appear exclusively in the so-called secondary colonies although all varieties of colonies do not contain them. The probable occurrence of asci and related special growth-forms in the tissues of the host offers a probable explanation for the recrudescence of the disease after apparent cure.

Demonstration of ascus formation suggests the allocation of these organisms among the ascomycetes rather than with the Oidia or Cryptococci.

Koser, S. A., and Galt, R. H.: The Oxalic Acid Test for Indol. Jour. Bacteriol., April, 1926, xi, No. 4, p. 293.

The authors extol the advantages of the oxalic acid test for indol first described by Pittaluga, in 1908.

Absorbent paper or filter paper is dipped in an aqueous solution of oxalic acid, dried, and cut into strips and a strip of the paper suspended from the cotton stopper in the mouth of the tube containing the culture to be tested.

As large a surface as possible should be exposed, but the paper must not come into contact with the culture.

If indol is formed it volatilizes at either room or incubator temperature and the oxalic acid paper becomes pink.

In the absence of indol the paper remains white.

The test has several advantages:

1. Nonvolatile compounds related to indol are eliminated.
2. The culture is not destroyed.
3. Repeated tests can be made.
4. Tests can be applied to cultures on solid media.

The test has not given false positive reactions and is fairly delicate though not so delicate as the Goré test.

Magoon, C. A.: Studies upon Bacterial Spores. I. Thermal Resistance as Affected by Age and Environment. Jour. Bacteriol., April, 1926, xi, No. 4, p. 253.

Studies were conducted upon spore suspensions of Bacillus mycoides.

The following method was developed for the preparation of the spore suspensions:

Clean, fine quartz sand, such as is used in greenhouse experiments, was passed through a standard 40-mesh brass sieve, subjected to thorough washing, and dried. Twenty-five gram quantities were then measured into 15 x 100 mm. Petri dishes, distributed over the bottom of the plate in an even depth, and sterilized in the dry air hot oven.

The sand serves as the substratum for the culture.

A suspension of spores from an old agar culture is made in standard beef extract peptone broth (Manual of Methods for Pure Culture of Bacteria, 1923), and boiled. With a sterile pipette and aseptic precautions, just enough of this suspension was transferred to the sand plates to exactly saturate the sand, and the cultures thus prepared incubated at 37° C.

The spores were thus suspended in a highly favorable medium, and maximum aeration was assured.

Hourly observations were made to determine the length of the spore cycle.

For the thermal resistance tests standard suspensions were prepared as follows:

About 20 c.c. of sterile distilled water was transferred aseptically to a sterile 20 x 150 mm. culture tube. With a freshly flamed small, flat-edged metal scoop a quantity was introduced into the tube which is then plugged, and agitated until a sufficient number of spores have been freed into the liquid. The suspension is then transferred with sterile precautions to a sterile centrifuge tube and centrifuged at high speed. The sediment is resuspended in sterile water and again centrifuged; this washing being twice repeated. The washed spores are finally suspended in sterile distilled water and standardized by opacity.

The standardized suspension is then transferred with aseptic precautions to a small sterile shell vial inserted into modeling clay at a convenient angle.

Pyrex tubing with an internal diameter of 4 mm. was drawn into capillary tubes about 9 to 10 cm. long with an internal diameter of 1 to 1.5 mm., and sealed.

A sufficient number of such tubes were placed in a glass Stender dish and covered with alcohol. As needed, a tube was removed with flamed forceps, the alcohol burned off, and the sealed ends clipped off by a sterile instrument. The tube is then dipped into the spore suspension, filled by capillary attraction, and resealed, the fluid being first centered to leave an air space at each end. The sealed and filled tubes were then dropped into cold potassium bichromate solution to sterilize the outside.

The solution is then washed off in cold water and the tubes placed in fresh alcohol chilled by placing the dish on crushed ice. Any tendency toward germination with resultant loss of heat resistance is thus obviated.

Heat resistance was tested by exposure in an oil bath, each test being made with five such tubes which, after removal from the bath, were placed in acetone in a 4-ounce salt-mouth bottle to remove the oil. They are then placed in alcohol until inoculated.

The sterility tests were made by flaming the plug and mouth of the culture tube in the usual way and then by introducing the capillary tube containing the spore suspension. In preparing the capillary tubes for this inoculation they were withdrawn from the alcohol with sterile forceps and, without flaming, one sealed tip was removed with the freshly flamed clipper. The open end was then inverted over the mouth of the culture tube from which the cotton plug has been removed, and the upper tip of the capillary snipped off. At the same instant the capillary tube was released and dropped into the nutrient broth of the culture tube. The plug was then replaced, and after making certain that the contents of the capillary had been forced up into the medium by the bubble of air formed when the tube touched the medium, the culture was ready for incubation.

A careful analysis of the experimental data presented leads to the following conclusions:

1. The bacterial spore is not dormant under ordinary conditions, as has commonly been supposed, but is, instead, sluggishly active.

2. The resistance of spores to heat is not a fixed property but a variable one, the degree of resistance being influenced by age, the temperature and humidity of the environment, and possibly by other factors.

3. The highest resistance to heat develops under conditions of moderate temperature and humidity, and is probably reached by the time the spores are sixty days old. Spores of different species of bacteria may be expected to vary somewhat in this respect.

4. Change in resistance takes place most slowly when spores are dry and cold, but low temperature accompanied by high humidity results in the development of a high degree of resistance.

5. In determining the thermal death points of spores that are to serve as the basis of processing schedules for canned foods the bacteriologist must take into account the change in resistance of spores under various conditions, and be as certain as possible that the resistance shown by the test spores represents the highest degree attainable by them.

The paper includes an extensive bibliography.

St. John, J. H.: Practical Value of Examination for Endameba Histolytica by Culture. Jour. Am. Med. Assn., April 24, 1926, lxxxvi, 1272.

Medium.—Four whole eggs are broken into a sterile receptacle. Fifty cubic centimeters of Locke's solution (NaCl, 9 gm.; CaCl, 0.24 gm.; KCl, 0.42 gm.; sodium bicarbonate, 0.2 gm.; glucose, 1 gm.; distilled water, 1,000 c.c.), are added and the whole emulsified.

Place about 10 c.c. of the emulsion in sterile tubes, incline, and heat to about 70° C. until solidified. Sterilize in the autoclave for twenty minutes at fifteen pounds pressure. To the solid medium now add sterile, inactivated human or horse serum diluted seven times in volume with sterile Locke's solution. The liquid layer should cover the whole or part of the slant.

Inoculate by rubbing against the wall of the tube a selected sample the size of a pea.

The medium must have been inoculated to insure sterility and should be warmed in the incubator before use.

Incubate at 37.5° C. and examine by taking a drop of fluid from the bottom of the tube.

By this method ameba have been grown from specimens transmitted by mail (forty-eight hours) and after eight days in the ice box.

Glenny, A. T., Pope, C. G., Waddington, H., and Wallace, N.: The Antigenic Value of the Toxin-Antitoxin Precipitate of Ramon. Jour. Path. and Bacteriol., January, 1926, xxix, 31.

A report of studies upon the antigenic value of the precipitate which occurs when diphtheria toxin and antitoxin are mixed, the following observations being recorded:

1. An emulsion of the toxin-antitoxin precipitate was equally efficient in doses of 0.001, 0.1, or 1 c.c., thus suggesting that the antigenic value of the precipitate depends upon the rate of dissociation of the toxin-antitoxin complex after injection.

2. The destructive action of heat is greater on toxin containing 0.5 per cent phenol than on uncarbolized toxin. The presence of trikresol also increases the rate of destruction by heat.

3. Neutral mixtures of toxin and antitoxin may become toxic upon evaporation because the increased concentration of phenol destroys antitoxin at a greater rate than toxin.

4. The addition of 0.2 per cent formaldehyde destroys half the antitoxic value of a serum.

5. The antigenic value of toxoid is slightly increased when precipitated by the addition of 1 per cent glacial acetic acid. Toxin or toxoid may be precipitated by the addition of varying quantities of potassium alum. An emulsion of such a precipitate has a high antigenic value.

6. Some batches of toxoid of high antigenic efficiency may fail to flocculate but the absence of flocculation does not necessarily indicate the absence of combining power.

Julianelle, L. A., and Reimann, H. A.: The Production of Purpura by Derivatives of Pneumococcus: I. General Considerations of the Reaction. Jour. Exper. Med., January, 1926, xliii, 87.

A study of the nature of the hemorrhagic purpura produced in white mice by the injection of pneumococcus extract.

If pneumococcus extracts are injected into white mice, within four to six hours the skin over the feet, tails, ears, snout, and genitals take on a dark bluish-purple color most marked where the hair is naturally scanty or absent.

There are no signs of intoxication, the reaction reaching its maximum in twenty-four to forty-eight hours and vanishing in five to seven days.

The reaction follows any method of injection but does not follow feeding the extract.

The purpura-producing principle resists heating to 100° C. for ten minutes; resists oxidation; is filter-passing; and is destroyed by trypsin.

It is obtained from pneumococcus extracts by full saturation with ammonium sulphate after the acetic acid-precipitable substances are removed.

It is common to all types of pneumococci and bears no apparent relation to virulence and is probably a degradation product of pneumococcus and is not associated with pneumococcus hemotoxin.

Reimann and Julianelle (II. The Effect of Pneumococcus Extract on the Blood Platelets and Corpuscles. Jour. Exper. Med., January, 1926, xliii, 87) found that the injection of the extract caused a marked reduction in the number of blood platelets and that purpuric lesions usually developed when the blood platelets dropped below 500,000 per c. mm.

The red cells were also reduced in number, but their destruction and regeneration were somewhat slower. The leucocytes were affected only slightly, if at all.

The extracts were both thromboiytic and hemolytic. Heated extract produced purpura but not anemia. Extracts adsorbed with blood or platelets showed a decreased thrombotic and hemolytic activity but were still able to produce purpura as well as severe anemia and thrombopenia.

Gordon, J., and M'Lead, J. W.: Inhibition of Bacterial Growth by Some Amino-Acids and Its Bearing on the Use of Tryptic Digests as Culture Media. Jour. Path. and Bacteriol., January, 1926, xxix, 13.

These studies are concerned with the effect of the addition of amino-acids to ordinary peptone broth, upon the growth of bacteria difficult to cultivate.

Fourteen different amino-acids were studied, as a result of which it was concluded that, in the absence of serum, etc., medium with a basis of tryptic digest are inferior to peptone broth for growing delicate bacteria, but that such media can be improved if a considerable part of the amino-acid is removed by butyl alcohol extraction.

Perlzweig, W. A., and Keefer, C. S.: The Immunization of the Pneumococcus: III. The Purification of the Water-Soluble Antigen. Jour. Exper. Med., December, 1925, xlii, No. 6, p. 747.

Actively immunizing fractions of protein nature have been isolated from broth cultures of pneumococcus Type I by ultrafiltration, precipitation at a definite hydrion concentration, and the separation of a soluble picrate fraction. The method appears to be suitable for the initial purification of this antigen.

Kasanin, J., and Grabfield, G. P.: Blood-Sugar Curves in Epidemic Encephalitis. Arch. Int. Med., January, 1926, xxxvii, 102.

Twenty-four blood-sugar curves were studied in seventeen cases diagnosed as epidemic encephalitis or its sequelae.

The curves were found to vary from the normal with such frequency that it seems probable that there is a fundamental disturbance of the sugar metabolism in this disease and during its mental sequelae.

Feinberg, S. M., and Lash, A. F.: Blood Calcium in Eclampsia. Surg., Gynec. and Obst., February, 1926, p. 255.

One of the many theories advanced as to the etiology of eclampsia ascribes this condition to hypocalcemia. Feinberg and Lash report their studies on this subject.

Method.—The determination of calcium was done according to the method of Kramer and Tisdall. Blood was drawn from the arm and the serum separated. Whenever possible 5 cubic centimeters of serum were used in the determinations. To the serum in a 15 cubic centimeter centrifuge tube was added one-half its volume of a 3 per cent solution of ammonium oxalate. This was allowed to stand until the following day. The sides of the tube were then rubbed with a rubber-tipped glass rod. The tube was centrifuged at high speed for about ten minutes, the liquid carefully decanted, distilled water added, and centrifuged again. This washing process was repeated three times. To the washed sediment were added 5 cubic centimeters of normal sulphuric acid and the tube kept at a temperature of 75° C.

This solution was titrated with a one-hundredth normal solution of potassium permanganate. The end-point was considered that point at which a faint pink remained over fifteen seconds. The calculations to be used are based on the fact that each cubic centimeter of permanganate solution represents 0.2 milligram of calcium.

The average blood calcium in 11 cases of normal pregnancy was 10.94 mg. per cent.

In 12 cases of eclampsia and preeclampsia, the average value was 10.21 mg. per cent.

In 4 cases of various conditions simulating eclampsia (uremia, chronic nephritis, epilepsy, and cavernous sinus thrombosis), the average value was 9.55 mg. per cent.

It is concluded that, despite the theoretical decrease in calcium to be expected in eclampsia, there is no appreciable relation between the blood calcium and eclampsia.

Murphy, W. P.: An Easy Method of Estimating the Amount of Jaundice by Means of the Blood Serum. Boston Med. and Surg. Jour., Feb. 18, 1926, exciv, 297.

Murphy prepares a set of standards for the estimation of the icterus index, thus eliminating a colorimeter.

The dilutions of the potassium bichromate standard and the equivalent "index" figures follow:

1:10,000	= 1	1:500	= 20
1:5,000	= 2	1:400	= 25
1:2,000	= 5	1:200	= 50
1:1,000	= 10	1:133	= 75
1:666	= 15	1:100	= 100

Mueller, J. H.: A Chemical Study of the Specific Elements of Tuberculin. Jour. Exper. Med., January, 1926, xliii, 1.

A study concerned with the protein or nonprotein nature of the substance in tuberculin to which is due its specifically toxic reaction in the body of the tuberculous animal.

A satisfactory conclusion as to the chemical nature of the fraction in question has not been formulated, but certain points in connection with the chemistry of "old tuberculin" have been demonstrated. It is concluded, as a result of the studies made, that the specific precipitin reaction and the skin reaction given by old tuberculin are attributable to two separate substances present in this material. The cause of the precipitin reaction is a nonprotein gum. It is also suggested that methods for the standardization of tuberculin by precipitin or complement-fixation reactions should be revised.

In a second paper **(The Preparation of Residue Antigens from Old Tuberculin.** Jour. Exper. Med., January, 1926, xliii, 9) Mueller describes the preparation in detail of the nonprotein gum responsible for the precipitin reaction. This material has been isolated from broth filtrates of human tubercle bacilli. It fixes complement and precipitates in high dilution in the presence of homologous immune serum, but fails to give a skin test in tuberculous animals.

Greenbaum, S. S.: Error of Basing Serum Diagnosis of Syphilis on the Kahn Reaction Alone. Jour. Am. Med. Assn., April 24, 1926, lxxxvi, 1273.

Greenbaum presents a number of cases in which parallel Kolmer and Kahn tests were made, from a study of which he utters a warning against using the Kahn test as the sole method of serologic study of syphilis and believes that in the best interests of the serum diagnosis of syphilis both tests should be employed.

Manwaring, W. H., and Shumaker, P. W.: The Relation of Anaphylaxis to Immunity. Jour. Am. Med. Assn., April 24, 1926, lxxxvi, 1271.

The relation of acquired hypersusceptibility to acquired immunity which has always been the subject of discussion among serologists, has narrowed down to a question of the identity or nonidentity of the specific precipitin and the so-called specific sensitizing body.

Many believe that these are identical and that small amounts of precipitin give hypersusceptibility, larger amounts conferring immunity.

The authors have studied this question in passively immunized dogs.

In dogs anaphylactic and immune sera usually gave approximately the same precipitin titer.

If a normal dog after exsanguination, is transfused from an anaphylactic donor, the animal becomes typically hypersensitive.

If the same procedure is followed except that the donor is an immunized animal, passive hypersusceptibility does not occur.

It is concluded, therefore, that in the dog the sensitizing antibody and the immune antibody apparently have wholly different physiologic properties.

Paullin, J. E.: Glucose Utilization in Renal Glycosuria. Trans. Assn. Am. Physicians, 1925, xl, 131.

A study of the results of the administration of glucose on the respiratory quotient, total metabolism, blood sugar, and glucose excretion in four cases of renal glycosuria.

In three cases 100 gm. were administered regardless of weight; in one case 1.75 gm. per kilo.

In all instances the response was similar to that of the normal individual and it would seem, therefore, that individuals with renal glycosuria metabolize and store carbohydrate the same as normal individuals and, so far as the evidence points, these patients do not develop diabetes mellitus.

Goldzieher, M., and Peck, S. M.: Granuloma Inguinale, Studies on the Etiology and Pathology. Arch. Path. and Lab. Med., April, 1926, i, 511.

Studies conducted on seven cases are reported together with a detailed description of an organism isolated from all cases—B. venerogranulomatis—producing complement-fixing bodies, allergic tests, and granulomatous lesions in rabbits.

The organism grown is illustrated in seven microphotographs and minutely described.

The organism grows best at 37° C., but growth will proceed at room temperature. Growth is most luxuriant under aerobic conditions, but occurs anaerobically.

Sugar Reactions.—Dextrose, galactose, inulin, lactose, maltose and saccharose are acidified with the production of gas. The strains vary in their acid and gas-producing qualities.

Hemolysis was absent; no indol production occurred; gelatine was liquefied; milk coagulated, with acid production.

On 4 per cent maltose-peptone agar, plate cultures at 38° C., there are seen fine surface colonies in from twenty-four to forty-eight hours. Some strains show no growth until they are forty-eight hours old. All strains reproduce themselves abundantly in twenty-four hours on subculture. On solid mediums there are formed small, round, transparent discs having a bluish opalescence, coalescing in older cultures. The periphery of the colonies is homogeneous, while the center shows a fine granulation. Confluent colonies have a wavy border. In reflected light the colony is gray, but the center of older ones shows a yellowish color and a loss of transparency. The sticky, mucoid growth, so characteristic of B. mucosus, was not observed.

The cultures have a peculiar, sour, fetid odor, like that produced by the lesions.

The growth characteristics on blood agar and rabbit blood agar do not differ from those observed on maltose-peptone agar. There is no hemolysis.

Plain and glucose broth become diffusely clouded at the end of from twenty-four to forty-eight hours. Later the growth settles to the bottom of the tube, forming a whitish sediment. In older cultures it assumes a stringy quality and adheres somewhat to the sides of the tube. There is no surface pellicle.

On nutrient and ascitic agar, growth differs in no way from that described.

On potato, a heavy gray smear is formed, which later becomes brown and colors the edge of the potato black.

The typical forms are best seen in smears from a twenty-four to forty-eight hour growth on Sabouraud's medium. The organisms are from 1 to 6 microns in length; the

smallest resemble cocci, diplococci, or small bacilli; the larger show a bacillus form that tapers to a point at one end, and contain one, two, or three granules. The large bacillus forms which we consider most typical are gram-negative. The smaller forms, especially the coccoid and cocco-bacillus types, are sometimes gram-positive. The inclusion bodies in the large forms are also many times gram-positive. The staining reaction does not depend on the culture medium; nor could any other factor apparently be found to explain the remarkable pleochromism.

The inclusion granules are different from the so-called polar bodies or any other known intracellular granules. They cannot be stained by special polar body staining methods, such as Abbott's and Moeller's. They do not show metachromatic staining. They are probably intensely stained parts of the bacillar substance, forming two or three round inclusions, often connected with a fine axial thread. Some of the bacilli contain but one coccoid body showing frequently a fine tail-like process. The body of the bacillus is often so faintly stained that it surrounds the darker staining granules like a halo.

Capsules are not formed. Pleomorphism is extreme. Spores or pigment are absent.

The cultures often have a peculiar odor like that of rancid fat or sour sweat due to the presence of fatty acids and neutral fats.

Leendertz, G.: The Conduct of the Protein Corpuscles as a Reflection of Certain Diseased Processes in the Human Organism. Klin. Wchnschr., Jan. 29, 1926, v, 175.

Whatever the theories may be to explain the changes in the plasma and serum characterizing those diseases associated with protein destruction, the fact remains that increased protein destruction is accompanied by an increased lability of the globulin compounds.

A method is described for measuring the proportion between the coarsely dispersed or labile globulin and the degree of stability of the plasma and serum.

The refraction exponent of the serum is first determined with a Pulfrich refractometer.

One c.c. of serum and 10 c.c. of freshly prepared 0.025 per cent acetic acid solution are placed in a centrifuge tube graduated at its tip at 0.5 c.c.

The labile globulins flocculate. The tube is centrifuged for ten minutes at 3000 r. p. m.

The limpid liquid is carefully removed to the 0.5 c.c. mark. The pipette must not touch the walls of the tube, the fluid being saved.

A further 1 c.c. of serum is added and carefully stirred with the precipitate by means of a thin glass rod, taking care to include the globulin fractions on the walls of the tube. As soon as the solution is entirely clear, one drop is placed on the prism of the refractometer and the refraction index (R_1) read.

During this time 1 c.c. of serum is mixed in a test tube with 0.5 c.c. of the liquid removed from the globulin sediment and the refraction index of this solution determined (R_2).

The difference between R_1 and R_2 multiplied by the dilution (1.5) is the numerical expression of the precipitable globulins in 1 c.c. of serum.

To find the measure of the stability of the serum the quotient is divided by the general protein content (R_S), this quotient representing the percentage of labile globulins in the general protein content of the serum.

$$\frac{(R_1 - R_2) \times 1.5}{R_S} = \frac{Q}{100} \text{ gives}$$

$$Q = \frac{(R_1 - R_2) \times 1.5}{R_S}$$

Or, as the author suggests:

$$\frac{\text{Labile globulin}}{\text{Serum protein}} = \text{quotient.}$$

McJunkin, F. A.: Identification of Three Types of Mononuclear Phagocytes in the Peripheral Blood. Arch. Int. Med., December, 1925, xxxvi, 799.

An investigation of the mononuclear phagocytes classified as "large mononuclear leucocytes" or "transitional cells."

The investigation was undertaken, first, to determine the occurrence in the blood of hemendotheliocytes and lymphendotheliocytes; and second, the relationship of these two phagocytes to the mononuclear benzidin reacting phagocytes of human blood.

METHODS

Method A. Phagocytosis in Vitro.—To 2 c.c. of 3.8 per cent sodium citrate solution in a graduated centrifuge tube, 3 c.c. of blood is added. The citrated blood is mixed with 1 drop of India ink (Higgins') and the mixture incubated at 37° C. for ten minutes. The mixture is centrifugated at low and then at high speed and the tube, after the careful complete removal of the supernatant liquid, returned to the incubator for fifteen minutes. The leucocytic layer is removed with a capillary pipette and smears are made on slides. It is essential to spread out completely the droplet and to guard against the smear reaching the edge of the slide; otherwise, most of the leucocytes may be lost. The phagocytosis obtained with human blood is quite complete but is very much less satisfactory in the case of the guinea pig and the rabbit.

Method B. Peroxydase Staining of Smears with Benzidin.—The fresh preparation of the leucocytic layer is covered for from thirty to sixty seconds with about 10 drops of an alcoholic solution of benzidin consisting of 100 mg. of dry benzidin dissolved in 25 c.c. of acetone-free 80 per cent methyl alcohol that contains 1 drop of hydrogen peroxide. Ten drops of distilled water are added to the alcoholic solution and two minutes are allowed for the reaction to take place. In the case of guinea pig or rabbit blood the reaction requires from five to ten minutes. Smears of guinea pig or rabbit blood must be treated as soon as they become dry, but the leucocytes of human blood react after several hours. The solution is washed off and hematoxylin (Harris' without acetic acid) applied for from twenty to sixty seconds. The hematoxylin is followed by 0.01 per cent eosin solution for twenty seconds. The preparation may also be stained by allowing Wright's stain, properly diluted, to act for from five to ten minutes.

Method C. Peroxydase Staining with Benzidin in Paraffin Sections.—The tissue, fixed for one day in 4 per cent formaldehyde solution, is cut into small bits not more than 2 mm. in thickness, placed in 70 per cent acetone solution for two hours, pure acetone for thirty minutes, benzene for twenty minutes, and paraffin at 52° C. for twenty minutes. Thin sections are attached to slides by allowing them to dry overnight at room temperature. The paraffin is removed with benzene (ten seconds) and acetone (ten seconds). The sections are covered with the diluted benzidin solution for five minutes, washed and stained with hematoxylin and eosin, as in Method B. After the eosin is washed off the excess of water is carefully removed with a soft cloth and the preparation dehydrated with acetone (ten seconds) and benzene (ten seconds); when it is mounted in balsam. To dehydrate in so short a time the solutions are run over the preparations from dropping bottles.

Method D. Supravital Staining of Leucocytes with Neutral Red.—In a centrifuge tube, to 10 c.c. of a neutral red solution consisting of 19 parts of saline solution and 1 part of saline solution saturated with neutral red (Gruebler), 2 or 3 drops of the fresh leucocytic layer without ink is added. After being mixed and allowed to stand for about ten minutes the cells are sedimented in the centrifuge tube and all of the supernatant liquid except about 2 drops is removed. With a platinum loop the cells are mixed with the liquid and a small droplet is transferred to the center of a cover glass rimmed with petrolatum and at once inverted on a slide for examination with oil-immersion lens. The cells are permitted to remain in the citrate the shortest time possible.

Method E. Zenker-Formaldehyde Solution (Formol) Method of Mordanting the Supravital Dye in Smears.—The reaction has usually reached the maximum intensity after about forty-five minutes contact with the dye. Heavy smears are made and just as drying becomes complete they are dropped into a solution consisting of 5 parts of 40 per cent formaldehyde and 95 parts of Zenker's fluid without acetic acid. After two hours' fixation the preparations are washed and at once covered with hematoxylin (Harris' without acetic acid) for two minutes. The hematoxylin is washed off and the slides immediately blotted dry. The dye granules are well preserved, but the nuclear staining is faint.

Method F. Iodine Vapor Method of Mordanting the Supravital Dye in Smears.—Tissue cultures stained supravitally have been successfully fixed in iodine vapor for microscopic examination and photographic purposes by Lewis. The smears of the fresh leucocytic layer, as they become dry, are placed for thirty minutes above iodine crystals in a glass jar 4 inches (10.1 cm.) in diameter and 6 inches (15.2 cm.) high with a ground-glass cover. They are removed from the iodine vapor, covered with hematoxylin (Harris' without acetic acid) for one minute, washed and at once blotted dry with filter paper. The nuclei are well stained with hematoxylin, but the dye granules are apt to range from brown to black. Lewis does not mention using a nuclear stain.

Method G. Zenker-Formaldehyde Solution (Formol) Method of Preserving Supravital Stains in Paraffin Sections.—Under anesthesia or immediately after removal from the living body a saline solution saturated with neutral red (Gruebler) is injected into the lymph nodes, spleen, or liver until the tissue becomes distended by the liquid. A second injection after a few minutes is advisable. After a half hour the tissue is placed for twelve hours in Zenker-formaldehyde solution (formol), consisting of 15 c.c. of 40 per cent formaldehyde and 85 c.c. of Zenker's fluid without acetic acid. It is then cut into pieces not to exceed 3 mm. in thickness and transferred to Zenker's fluid without acetic acid for from twelve to twenty-four hours. The bits of tissue are then placed in pure absolute acetone for one hour (two changes), in benzene for twenty minutes, and in paraffin at 52° C. for twenty minutes. Extra thin sections are then attached to slides with albumin fixative by allowing to dry overnight at room temperature. To stain, the paraffin is removed with xylene (ten seconds) and pure acetone (ten seconds). After immersion in water (five seconds) the slide is stained very lightly with hematoxylin (Harris' without acetic acid) for about five seconds. The section is then dehydrated with pure acetone for ten seconds, at once covered with xylene for ten seconds, and mounted in balsam. To limit the action of the acetone and xylene to these times, the slides are stained singly and the solutions run over them from dropping bottles.

CONCLUSIONS

1. Mononuclear benzidin-positive phagocytes reacting to neutral red supravital staining are present in normal human blood, occasionally in guinea pig blood, but not in rabbit blood. Since they are found only in bone marrow and spleen they probably originate in these tissues.

2. Lymphendotheliocytes, benzidin-negative mononuclear phagocytes reacting to supravital staining with neutral red (occasionally with the formation of granules in rosettes), are present in the normal peripheral blood of guinea pigs, rabbits, and man. They arise from the lymphatic reticuloendothelium and are transformed into the epitheliod cells of tubercles.

3. Hemendotheliocytes, benzidin-negative mononuclear phagocytes not reacting to supravital staining with neutral red, probably arise from the blood vascular endothelium and do not occur in the normal peripheral blood.

McCrackan, R. F., and Passamaneck, E.: Manganese in the Urine, its Detection and Determination. Arch. Path. and Lab. Med., April, 1926, i, 585.

To 100 c.c. of urine in a Kjeldahl flask, 20 c.c. of concentrated nitric acid is added. If the urine contains less than 1 mg. of manganese per liter of urine, larger amounts in the same proportion are used. This is followed by evaporation to a paste on a sand bath; after cooling, 5 c.c. of concentrated sulphuric acid is added. The preparation is then heated at high temperature until about a third of the acid is driven off as heavy white fumes. With urine high in phosphates, or when a large quantity of urine is used for the analysis, the amount of sulphuric acid may have to be increased. After cooling, 5 c.c. of concentrated nitric acid is added, followed by heating until brown fumes disappear. If oxidation does not seem to be complete, this step is repeated again and again until there is no doubt, more sulphuric acid being added if necessary. After cooling, transfer is made to a 100 c.c. volumetric flask by means of about 75 c.c. of distilled water. Then 5 c.c. of concentrated nitric acid, 1 c.c. of tenth-normal silver nitrate and 1 c.c. of 50 per cent ammonium persulphate are added and diluted to the mark. One or more standards are prepared with similar

amounts of reagents from manganous sulphate or manganous nitrate, or potassium permanganate in 100 c.c. flasks, and both the known and the unknown are heated at the same time in the water-bath until the formation of permanganic acid is complete. If the depth of color in the unknown is deep enough, comparison is made in the colorimeter, or in Nessler tubes if it is too faint. When manganese is found a blank test should be made on the reagents as a control.

In case the acidity is already quite high due to using more than the amount of sulphuric acid recommended, less nitric acid may be used before adding the persulphate, or its use may be omitted. In case much silica from the glassware is present, it may be ignored until after the solution is made up to volume and the color developed. Then it may be removed from a portion by centrifuging, or it may be allowed to settle by gravity.

The test is so sensitive that great care must be taken to prevent contamination.

The amount of manganese in one normal urine has been found to be less than 1 part in 50,000,000.

Jaffé, R. H.: Studies in Vital Staining in Experimental Tuberculosis. Am. Rev. Tubere., February, 1926, xiii, 97.

Most intensive vital staining of rabbits with India ink or colloidal iron does not prevent the formation of tubercles. This observation is not in accordance with the possibility of a functional elimination of cells by the granular storage of foreign material.

The decrease of the dye in the growing tubercle, and its absence under certain conditions in larger tubercles, is due to lack of proper circulation rather than to diminished vitality of the epithelioid cells.

Schuback, A.: The Bacteriologic Investigation of Material Containing Proteus. Klin. Wchnschr., January 8, 1926, v, 67.

For the culture of material containing B. proteus, Schuback uses nutrient agar to each 10 c.c. of which has been added 0.2 to 0.3 c.c. of proteus agglutinating serum diluted 1:1 with 0.5 per cent phenol. The serum is prepared by the immunization of rabbits and should be of not less than 1:6,000 titer.

By this procedure the tendency of proteus organisms to overrun poured plates is inhibited.

Balint, M.: Buffered Water for the Romanowsky-Giemsa Stain. Klin. Wchnschr., January 22, 1926, v, 147.

After the blood smears have been air dried they are fixed for five minutes with Jenner's solution and prestained for ten minutes by the addition of 2 c.c. of the following solution:

KH_2PO_4	9.078 gm.
$Na_2HPO_4(2H_2O)$	11.876 gm.
Boiled distilled water	100 c.c.

The smears are then washed with this buffered water, stained for twenty-five minutes with Giemsa (1 drop to 1 c.c. of buffered water) washed with buffered water and air dried.

Chanutin, A., and Guy, L. P.: The Fate of Creatine When Administered to Man. Jour. Biol. Chem., January, 1926, lxvii, 29.

As a result of careful experiments on two normal individuals the following conclusions are formulated:

1. The absorption of creatine from the alimentary tract appears to be complete. There is no evidence of its bacterial decomposition.

2. The creatinine content of the urine in man increases after the ingestion of large doses of creatine, the increase, apparently, being derived directly from the creatine fed.

3. Evidence is presented to indicate that creatine has an indirect action on nitrogen metabolism.

Haynes, R.: Modification of the French Azure C. Tissue Stain. Stain Technology, April, 1926, i, 68.

1. Xylol, 3 min.
2. Absolute ethyl alcohol, 3 min.
3. Ninety-five per cent ethyl alcohol, 3 min.
4. Water, 3 min.
5. One and five-tenths per cent aqueous solution Azure I, 5 min.
6. Absolute ethyl alcohol, 5-10 seconds. (For formalin fixed material use 3 per cent glacial acetic acid in absolute alcohol.)
7. Saturated solution alcohol soluble eosin (ethyl eosin) in clove-oil, 30 seconds.
8. Xylol, 10-30 seconds.
9. Xylol, 1-2 min.
10. Xylol, 1-2 min.
11. Xylol balsam.

Young, C. C., and Orr, P. F.: Dosage of Toxin for Active Immunization against Scarlet Fever. Jour. Am. Med. Assn., May 1, 1926, lxxxvi, 1340.

Preliminary report of an attempt to determine whether as great an immunity can be produced with three as with five doses of scarlet fever toxin.

The administration of three doses in a group of twenty-four individuals—500, 5,000, and 30,000 skin test doses—at intervals of two weeks was found to be without injurious effects and to produce a satisfactory immunity as great or greater than that following five doses as usually advised.

Welker, W. H., Thomas, W. A., and Hektoen, L.: Urinary Proteins, Crystalline Proteins of Nephritis. Jour. Am. Med. Assn., May 1, 1926, lxxxvi, 1333.

In ten cases of nephritis, proteins were obtained in the form of globular crystals from the urine. In one instance the protein appeared as needle-like crystals.

Precipitin tests indicate that the protein crystals consist of compounds of serum albumin, euglobulin, and pseudoglobulin.

As a preliminary treatment, the urine was saturated to 25 per cent with ammonium sulphate, left standing for a few minutes until flocculation had taken place, and then filtered. The filtrate was returned to the filter paper until a clear fluid was obtained, which was completely saturated with ammonium sulphate. The precipitate was dissolved with the requisite amount of distilled water, giving a protein solution of moderate concentration. This solution was treated with saturated ammonium sulphate solution until a slight, but permanent, flocculent precipitate formed. The mixture was then filtered and refiltered until a crystal-clear fluid resulted. The solution was set aside in a crystallizing dish, and usually at the end of twenty-four hours a copious sediment had appeared, which under the microscope was composed of globular crystals of protein, as shown by appropriate tests. After standing for a number of days, but before any gross separation of ammonium sulphate had occurred, the solution was filtered, the sediment on the filter paper dissolved in distilled water, and again treated with saturated ammonium sulphate until a slight but permanent precipitate had formed. The solution was filtered and refiltered until clear, and again set aside for crystallization. Again globular crystals of protein appeared at the end of twenty-four hours.

Noguchi, H.: Abnormal Bacteria Flagella in Cultures, Their Resemblance to Spirochetes. Jour. Am. Med. Assn., May 1, 1926, lxxxvi, 1327.

Besides the leptospira-like filaments, probably originating from red blood corpuscles under certain conditions in vitro, other spiral elements exist which may be erroneously interpreted as spirochetes. These are the exaggerated detached flagella of certain bacteria, produced under cultural conditions. In cultural studies of microorganisms, the occurrence of

these spiral elements must be borne in mind, particularly in connection with dark-field illumination.

Spirochetes belonging to the spironema and treponema groups also produce, under certain cultural conditions, exaggerated flagellar appendages. These terminal flagella are similar in appearance and structure to the axial spiral filaments of the same organisms, but are much finer. The axial filaments are covered with a layer of cytoplasm which can be removed by the action of bile. The motility of these organisms resides in the portion of the filament next the attachment of the flagellum at either end.

The great resemblance which exists between the flagella of motile bacteria and the flagellar and axial spiral apparatus of certain spirochetes seems to indicate that the axial filaments are probably a modified apparatus of similar origin especially adapted to the locomotion of spirochetes, and therefore supports the hypothesis of a close phylogenic relationship between bacteria and spirochetes.

The paper is illustrated with twenty-five microphotographs.

Adams, S. F., and Brown, G. E.: The Blood in Cases of Hypertension. The Relationship between Anemia and Renal Insufficiency. Ann. Clin. Med., December, 1925, iv, 463.

In 76 cases of hypertension uncomplicated by gross loss of blood or by conditions obviously related to the production of anemia and in which renal function was adequate, the hemoglobin was more than 100 per cent in 60 per cent.

In 90 per cent of cases where renal function was inadequate the hemoglobin and red cells were diminished.

A parallelism seems to exist between erythrogenic and renal function in cases of hypertension. The recovery of the blood does not parallel the recovery of renal function.

The presence of anemia in cases of primary hypertension and consequent arteriosclerosis, in the absence of complicating disease or gross loss of blood is good evidence of existing or preexisting renal insufficiency.

Felty, A. R., and Heatley, C. A.: The Nasal Passages in Lobar Pneumonia. Jour. Am. Med. Assn., April 17, 1926, lxxxvi, 1195.

In a series of sixteen cases of lobar pneumonia, pneumococci of corresponding type to those found in the sputum were isolated from the middle fossae of the nose in every case.

The nasal passages in fifteen of the sixteen patients were examined with the nasopharyngoscope; of these, all showed hyperemia of the mucous membranes, and six had signs of acute suppurative sinusitis. In eight cases, anteroposterior roentgenograms of the sinuses were made; in seven patients, clouding of one or more of the sinuses was observed.

In this small series of patients, acute pneumococcal sinusitis was a frequent accompaniment of lobar pneumonia.

Lindsay, J. W., Rice, E. C., and Selinger, M. A.: Scarlet Fever. Jour. Am. Med. Assn., April 17, 1926, lxxxvi, 1191.

As a result of an analysis of cases of scarlet fever studied in the Garfield Memorial Hospital the following conclusions are presented:

1. The intracutaneous injection of properly concentrated scarlet fever toxin, after the method of the Dicks, properly controlled by use in groups, is a reliable means of determining susceptibility or immunity to scarlet fever.

2. This method is also frequently of definite assistance in diagnosis.

3. It is possible actively to immunize susceptibles for a considerable period.

4. Care should be used to adjust the dose of toxin for active immunization so as to avoid serious reactions in those who may have an unusually small amount of natural antitoxin, as indicated by an unusually large and intensely positive Dick reaction.

5. There is ample evidence that the serum prepared by the method of Dochez, now available for the treatment of scarlet fever, possesses true and specific antitoxic properties in effective concentrations.

6. The early use of sufficient amounts of antitoxin apparently reduced the incidence of complications of severe degree.

7. A single large dose of antitoxin will probably prove more satisfactory, from every standpoint, than a small or moderate dose with the possibility of repetition.

8. The intramuscular injection appears to be satisfactory in practically all cases, the intravenous possibly being indicated in critical conditions.

9. We suggest the advisability of testing all patients for sensitiveness to serum before giving the antitoxin, even though workers are not in complete agreement as to the importance of this procedure.

Herrold, R. D., and Saelhoff, C. C.: Skin Reactions with Filtrate of Koch Strain of Bacillus Tuberculosis. Jour. Am. Med. Assn., March 13, 1926, lxxxvi, 747.

The so-called Koch strain of the tubercle bacillus is an avirulent culture which gives a profuse growth overnight on the ordinary solid and fluid nutrient mediums.

Transplants from solid medium were made into nutrient broth containing 0.1 per cent dextrose and 0.1 per cent dibasic sodium phosphate in place of the sodium chloride of the ordinary broth. A four-day growth was passed through Berkefeld N filters, and the filtrate diluted with physiologic sodium chloride solution for skin tests. Skin reactions were obtained in normal adult persons in dilutions up to 1:50 injected intracutaneously in a quantity of 0.1 c.c. Tuberculin syringes were used and a 27 gauge needle. The injections were made in the forearm as a rule, but in persons with a thin skin the arm was satisfactory.

Within twenty-four hours the majority of apparently normal adult persons developed an area of redness at the site of injection, which varied from 1 to 3 cm. in diameter and reached its maximum in from twenty-four to forty-eight hours; after fading, there frequently is left a pigment spot which persists for several days and in some instances for several weeks. Such reactions are classified as positive.

A second type of reaction was smaller in size—from 5 to 10 mm. in diameter—and after forty-eight hours disappeared without leaving any pigmentation. This reaction is considered as doubtful.

The tests without reaction at the end of forty-eight hours are classed as negative. Controls of broth diluted with salt solution gave no reactions.

The accompanying table shows types of patients tested:

TYPE OF PATIENT	NUMBER OF PATIENTS	NUMBER OF REACTIONS		
		POSITIVE	DOUBTFUL	NEGATIVE
Advanced tuberculosis	35	0	0	35
Moderately advanced tuberculosis	31	0	0	31
Incipient tuberculosis	8	0	0	8
Apparently normal adults	61	47	8	6
Apparently normal children	14	2	2	9

Obviously, the interpretation of these results must be tentative, but the presence of a reaction in the majority of apparently normal adult persons seems to indicate that there is a substance produced by the growth of this strain of tubercle bacillus in broth which acts in a different way from tuberculin. The reaction to the filtrate in normal adult persons and the absence of reaction in tuberculous patients and in many children may mean that, in the presence of an active tuberculous focus, there may be sufficient antisubstance produced to neutralize the toxic substance.

Smith, D. C., and Gill, R. D.: Nonspecificity of the Luetin Test. Am. Jour. Syph., April, 1926, ix, 2.

After a study of the test in 17 syphilities and 68 nonsyphilities it was concluded that the lutein test is nonspecific and unreliable as a diagnostic procedure.

Poire, A. F., and Carranza, M. A.: Staining Methods for the Koch Bacillus. Semana méd., October 8, 1925, xxxii, 877.

The authors, after a comparative study of various methods, recommend the following:

1. Fix slowly by heating, at a temperature not exceeding 60° to 70° F.

2. Pour on the staining agent, drop by drop of carbolized fuchsin, until the entire specimen is covered.

3. Heat slowly until some vapor is given off.

4. Leave the specimen alone until the disappearance of vapors.

5. Heat again, until vapors are given off.

6. Repeat the fourth item.

7. Repeat the fifth item.

8. Repeat the fourth item.

9. Pour cold water on the specimen.

10. Wash with sufficient water under the water faucet; not too violently.

11. Decolorize with sodium sulphite 10 per cent, heated to 80° and newly prepared, which decolorizes in a few seconds.

12. Wash with an abundance of water.

13. Stain the background with methylene blue in dilute watery alcoholic solution, in a proportion of one drop per five cubic centimeters of distilled water.

14. Wash with an abundance of water.

15. Slant the specimen, so as to permit the fluid to drain off. Dry with filter paper then gently heat the slide. Examine.

The sodium sulphite used by the authors is crystallized and preserved in well-closed bottles, in order to guard against its transformation through the presence of air. The solutions are prepared just before using, because after twenty-four hours, the sulphite becomes changed and transformed into the sulphate, its power of decolorization diminishing until after three days it ceases to decolorize. The quantity that is prepared should be proportionate to its daily employment, and it is therefore advisable to make packages of two and five grams, which are kept in well-closed wide-necked flasks, ready to be dissolved in 20 or 50 c.c. of distilled water.

Patton, H. W., Blackford, S. D., and Smith, D. C.: Cutaneous Tests with Suspensions of Treponema Pallida. Med. Jour. and Rec., January 6, 1926, cxxiii, 4.

A study of 100 syphilitic and 60 nonsyphilitic patients from which the following conclusions are formulated:

1. Intradermal injections of saline suspensions of Treponema pallida are without value as a practical diagnostic test.

2. A suspension of Treponema pallida in twentieth-normal sodium hydroxide gives no better results than the saline suspension.

3. Potassium iodide in doses of 60 grains daily does not affect the results of intracutaneous tests with either solution.

Moritz, A. R.: The State of the Serum Calcium in Experimental Hypo- and Hypercalcemia. Jour. Biol. Chem., December, 1925, lxvi, 343.

In a general way, a decrease in serum calcium following thyroparathyroidectomy shows a disproportionately great decrease of the diffusible fraction; but in an increase in serum calcium produced by injection of parathyroid extract (Collip) the ratio of colloidal and diffusible calcium did not show consistent changes.

Regan, J. C., and Tolstouhov, A.: Significance of the Blood Chemical Changes in Pertussis. Jour. Am. Med. Assn., April 10, 1926, lxxxvi, 1116.

A total of 682 blood chemical analyses performed in cases of pertussis have given the following results:

1. There is a diminution of the total inorganic phosphorus associated with a lowering of the hydrogen-ion concentration of the blood, while the plasma bicarbonate remains within normal limits.

2. These changes occur early in the disease, appearing in the case of the inorganic phosphorus in the catarrhal stage.

3. Both alterations are well developed, especially the change in phosphorus, during the first few weeks of the paroxysms, and show a certain degree of parallelism in their course which signifies a close interrelation.

4. In moderate and severe cases treated with alkalis, the inorganic phosphorus rises steadily from the third week, while in untreated cases of the mild type, the rise does not begin until the sixth week. The same is true in a less decided way of a P_H value before, as compared to those during and after, treatment.

5. The diminution of inorganic phosphorus bears no relation to age, but only to the stage of the disease, and for reasons mentioned in the text has no underlying rachitic basis.

6. The calcium content, while exhibiting slight mobility, as the result, possibly, of shifting of calcium in connection with the characteristic phosphorus and P_H alterations, has no constant alterations of a distinct type.

7. These changes indicate an acidosis of an uncompensated type (Type 6, Van Slyke), which has as a cause the accumulation or increased concentration of free carbon dioxide in the blood. This laboratory observation is easily correlated with several of the symptoms so prominent in pertussis—the paroxysms, the vomiting, parenchymatous emphysema and convulsions.

8. The vomiting of the disease may be a compensatory mechanism adopted by the body to eliminate acid in an attempt to maintain a normal acid base balance.

9. This contention of an uncompensated acidosis is further substantiated by the effects on the disease of alkali therapy.

10. Alkalis administered early appear usually to abort the disease, and associated with the cure is a rapid rise of inorganic phosphorus and a change in P_H of the blood, while, if given late, cure supervenes in a relatively short period.

CONCLUSIONS

There occurs in pertussis an uncompensated acidosis, which is intimately connected with the pathogenesis of the paroxysms.

If the acid base unbalance is corrected, the clinical symptoms are quickly ameliorated, and the organism returns to normal.

Sheridan, W. F.: Rapid Paraffin Embedding of Tissue. Internat. Assn. Med. Mus. Bull., May 4, 1926, xi, 124.

Salt-mouth bottles of 200 c.c. capacity are loosely packed with a layer of filter or tissue paper and filled with acetone so as to provide a layer about one-half to three-quarters of an inch above the level of the paper. The pieces of tissue should not be over 3 mm. in thickness.

1. Boil tissue in neutral 10 per cent formalin for thirty seconds.
2. Blot.
3. Acetone two and one-half to three hours.
4. Blot.
5. Xylene fifteen to twenty minutes or until tissue is translucent.
6. Blot.
7. Paraffin 52° C. melting point, 4 twenty minute changes in 60° C. oven.
8. Embed and congeal with cold water.

Sheridan, W. F.: Rapid Hematoxylin-Eosin Staining Method for Paraffin Sections. Internat. Assn. Med. Mus. Bull., May 4, 1926, xi, 57.

The sections should not be over 5 microns in thickness. Attach the sections to the slides with Mayer's glycerin albumin and stain as follows:

1. Xylene two minutes.
2. Rinse with alcohol (95 per cent) from drop bottle

3. Hematoxylin (Harris) two minutes.
4. Rinse with alcohol (95 per cent) from drop bottle.
5. Acid alcohol (1 per cent HCl in 70 per cent alcohol) fifteen or more seconds.
6. Rinse with alcohol (95 per cent) from drop bottle.
7. Ammoniated alcohol (stronger ammonia water 4 drops, alcohol 95 per cent 50 mils) until rose color is replaced by blue—about one minute.
8. Rinse with alcohol (95 per cent) from drop bottle.
9. Eosin (eosin alcohol soluble 0.25 per cent in alcohol, 95 per cent) thirty seconds.
10. Rinse with acetone from drop bottle.
11. Xylene.
12. Mount in balsam.

The use of Harris' hematoxylin is essential for good results. The ammoniated alcohol should be prepared just before use and the rinsing with acetone should be thorough. The acetone should not give more than a faint turbidity on shaking with xylol (i.e., nearly water free).

The clearing in xylol after acetone is almost instantaneous.

Prince, L. H.: A Rapid Stain for Nerve Tissue. Internat. Assn. Med. Mus. Bull., May 4, 1925, xi, 55.

Fuchsin f. bac. (Gruber) saturated aqueous solution	gtt. 3-5
Erythrosin (Gruber) 1 per cent aqueous solution	gtt. 15
Methyl orange (Campbell and Bell) saturated aqueous solution	gtt. 40
Aniline blue porous (Campbell and Bell) saturated aqueous solution	gtt. 25

Mix in the order listed and permit to stand for twenty-four hours. Frequent agitation of the solution during this period is desirable as more perfect blending results. No precipitates are formed. Since the mixture appears to stand up well, multiples of the unit above may be mixed. Three or four times the unit will provide sufficient mixture for many slides. A dropper is preferred for accuracy in mixing.

The prepared slide is removed from the water and the excess shaken off. Sufficient stain to cover the section is added and permitted to act for ten to thirty seconds. Wash in running water, dehydrate in absolute alcohol (alcohol that has been dehydrated with anhydrous copper sulphate will serve the purpose and is much less expensive), blot carefully but quickly, clear in xylol and mount in balsam.

It is recommended that several sections of the same specimen be stained over periods ranging from five seconds to sixty seconds at five-second intervals, in order that the reactions of the many elements to the stain may be noted. This study will prove of value and serve as a guide for future use in demonstrating some one particular feature by selecting the time suitable for showing the desired object to best advantage.

In general the nerve cells stain light to very dark purple with deeper staining Nissl bodies fairly well seen. Normal cells usually stain deeply in twenty seconds. The nuclei are quite definitely blue with an orange-red nucleolus. In certain cells the nucleus is outlined and transparent but the chromatin fragments stand out sharply in deep blue.

The myelin varies from pink to deep red. Particularly does the latter tint prevail if this substance is granular. The axis cylinder is almost black, but reacts less intensely in proportion as it has degenerated until it takes the orange tint. This remark applies equally to the nerve cells which become mottled if degenerating and finally react to orange staining exclusively.

The neurolemma is well shown as a light or dark-blue tracery, depending on the duration of staining.

The neuroglia likewise reacts to the blue stain but shows quite well the detail within its structure if not overstained. The pathologic types are easily recognized.

The erythrocytes stain orange to red-orange in proportion to the time of stain contact. Lymphocytes stain a pale blue.

Corpora amylacea are pale blue.

An interesting and practical step in the technic consists of treating the section as it is taken from the first alcohol with a 1 per cent solution of glacial acetic acid in 90 per cent alcohol for ten to twenty seconds or longer as the worker wishes. The watery stain is applied directly to the section after the excess of the acid alcohol has been shaken off. The staining period is shortened but may be carried on for the full limit. The observer will note marked intensity in staining in some of the elements and this may prove of value in certain instances. The nerve-cell detail is obscured if too long staining is done. On the other hand the neuroglia is intensified as are all the connective tissues. The myelin is distinctly benefited by this treatment, and all red cells stain a bright orange.

Kohn, L. A.: Recurrent Type I Pneumonia. Jour. Am. Med. Assn., December 12, 1925, lxxxv, 1888.

Two attacks of Type I pneumococcus pneumonia occurred at an interval of six weeks in a person who had suffered from several previous untyped pneumonias. There was little response in the production of humoral antibodies to these attacks.

The first attack recorded was undoubtedly a lobar infection, and the isolation of pneumococci from the blood stream with the presence of organisms and abundant precipitinogen in the sputum establishes it as due to Type I. The second attack, while involving three separate lung areas in three lobes, was nevertheless croupous pneumonia, clinically, and the patches of consolidation appeared by the roentgen ray to be larger than those usually involved in lobular pneumonia. The sputum was twice injected into mice, and each time a pure culture of pneumococcus Type I was recovered. This organism was fatal to mice when injected intraperitoneally in quantities down to 10^{-5} c.c. of eighteen hour broth culture, and was agglutinated to the titer of Type I agglutinating serum (1:64). It is exceedingly unlikely that any other organism was responsible for the pneumonia.

Lundquist, R.: A Proposed Modification of the Kaiserling Method for Preserving Gross Specimens. Internat. Assn. Med. Mus. Bull., May 4, 1926, xi, 16.

SOLUTION I

Potassium acetate	85 gm.
Potassium nitrate	45 gm.
Chloral hydrate	80 gm.
Formaldehyde (40 per cent gas)	444 c.c.
Water	4000 c.c.

The technic for using the solution is the same as for other solutions. Specimens are placed in the fixing fluid as soon as possible. Ten to twelve times the volume of fixing fluid to the volume of the specimens is used, and the specimens are not allowed to lie against one another or against the bottom of the container. A container deep enough to permit suspension of the specimen by a string attached to a paraffined cork is used. Care must be exercised to attach the string in such a way that the suspended specimen will assume its natural shape. The use of the paraffined corks has the advantage of facilitating the removal of any specimen desired, and of allowing a larger number of specimens to be placed in one jar without the objectionable feature of one specimen pressing against another at the bottom of the jar. Specimens should be thoroughly fixed but should not be left in the fixing solution too long beyond this point. However, the danger of loss of color due to overfixation is not nearly so great with this method as with the original Kaiserling method. The time of fixation, of course, varies with each specimen. After fixation the specimens are thoroughly washed in running water to remove all formaldehyde. At this stage the specimens are trimmed and all specimens having cut surfaces are resurfaced. They are then placed in the following solution:

SOLUTION II

Potassium acetate	10 gm.
Chloral hydrate	5 gm.
Glycerin	10 c.c.
Water	90 c.c.

This solution is changed twice, the specimens remaining in the first solution about twelve hours. The colors become brighter in this solution and the consistency and color obtained are much closer to the original than by any other method tried. The selection of a preservative for the final mounting fluid to prevent the growth of yeasts, fungi, etc., is a very important factor in the preservation of the colors, as well as of the consistency of the specimen, and must receive still further study. There is much evidence that the use of arsenious acid for this purpose is preferable to formol, phenol or thymol, but this is still under trial.

Kohn, L. A.: Acute Mercuric Chloride Poisoning. Arch. Int. Med., February, 1926, xxxvii, 225.

Death from mercuric chloride taken by mouth may ensue within hours, apparently with circulatory collapse, with little renal damage and no evidence of uremia. Evidence is presented which suggests that direct myocardial damage may account in part, at least, for this early toxic death. In three severely poisoned cases, the white count reached 34,000 or higher in a few hours, and it is suggested that the degree of elevation of the leucocytes may be an index of the severity of poisoning, with an unfavorable prognosis when levels of from 30,000 to 40,000 are found. Last, while it is not denied that sodium thiosulphate may have value in treatment, it should be emphasized that it may fail to exert detoxicant action, and should not be administered to the neglect of established therapeutic methods.

Rosen, I., and Krasnow, F.: Blood Cholesterol Findings in Syphilis and in Other Skin Diseases. Arch. Dermat. and Syph., April, 1926, xiii, 506.

Report of a study of the blood cholesterol in a variety of conditions.

The blood cholesterol was low in 100 per cent of the patients with untreated primary syphilis, in 50 per cent of the untreated secondary cases, and in 25 per cent of the untreated tertiary cases.

After treatment all our primary cases showed a rise in the cholesterol content to normal or above normal, whereas some of the secondary and tertiary cases remained low.

The cholesterol content in 50 per cent of the pregnant syphilitic women showed a high cholesterol value, 35 per cent showed a normal value, and 5 per cent a low value.

The blood cholesterol content in two infants with active manifestations of syphilis was low.

The blood cholesterol content in 82 per cent of the treated patients who had congenital syphilis was normal.

There seems to be no direct connection, at least as far as these studies are concerned, between the cholesterol content of the blood and the results of the Wassermann reaction.

The cholesterol content of the blood was high in psoriasis and dermatitis venenata and normal in acne vulgaris, dermatophytosis and dermatitis seborrheica.

Berger, S. S., Cohen, M. B., and Sellman, J. J.: Liver Functional Tests; a Comparative Study of Five Methods in 100 Clinical Cases. Jour. Am. Med. Assn., April 10, 1926, lxxxvi, 1114.

All the tests were done either simultaneously or within forty-eight hours, the following being studied: Van den Bergh's, Widal hemoclastic, Rosenthal, examination of urine for urobilin and urobilinogen, and for bile salts.

In 10 cases of liver disease with jaundice due to stone or tumor the tests showed: Van den Bergh 100 per cent; Widal 40 per cent; dye 60 per cent; urobilin 30 per cent; bile salts in urine 80 per cent.

With 6 cases of liver disease without jaundice: Van den Bergh 83.3 per cent; Widal 50 per cent; dye 33.3 per cent; urobilin 66.6 per cent; bile salts in urine 33.3 per cent.

In 37 cases in which liver disease was suspected, the positive reactions were as follows: Van den Bergh 37.9 per cent; Widal 59.4 per cent; dye 16.2 per cent; urobilin 32.5 per cent; bile salts in urine 48.6 per cent.

In 38 cases in which liver disease was not suspected the results obtained were: Van den Bergh 42.1 per cent; Widal 39.4 per cent; dye 10.5 per cent; urobilin 5.5 per cent; bile salts 23.6 per cent.

As a result of the studies the following conclusions are advanced:

1. It is important to bear in mind that these tests represent different functions of the liver. Any one or more or all of these functions may become impaired. Again, one or more of these functions may escape injury. Therefore, the various tests do not give parallel results. When we attempted to separate clinical cases into groups of liver disease or no liver disease by means of any one of these tests, unsupported by other clinical evidence, we were unable to do so.

2. When all the tests were positive, we were dealing with liver disease, clinically of the most severe type; namely, toxic jaundice.

3. When all tests were positive except one; namely, four positive and one negative, clinical liver disease was present, usually of a chronic type, such as that seen in Banti's disease or pernicious anemia and cirrhoses.

4. In every case in which all the tests were positive except the Widal, there was obstructive jaundice due to tumor. This finding is of great value in differential diagnosis.

5. When only three tests were positive it was impossible to correlate the findings with the clinical picture, as there were many cases in which liver disease was suspected which did not give positive reactions to more than one or two tests, and conversely, there were many cases in which liver disease was unsuspected which gave as many positive results.

6. At present they are of use chiefly in the differential diagnosis and in following the progress of a given case. The greatest amount of information can be gained by doing all the tests simultaneously and repeating them often.

Wile, N. J., and Belote, G. H.: Syphilitic Alopecia, Its Relation to Neurosyphilis. Arch. Dermat. and Syph., April, 1926, xiii, 495.

A histologic study of 37 cases. Two distinct types are recognized: that occurring without visible accompanying syphilides, and that in which the loss of hair is apparently due to papular or other lesions on the scalp.

The authors conclude that:

1. Syphilitic alopecia of the essential type has a high associated incidence of meningeal syphilis, as indicated by spinal fluid findings.

2. The absence of the accepted criteria in the spinal fluid cannot, moreover, be accepted as absolute evidence of the absence of such involvement.

3. Microscopic study shows that the essential syphilitic alopecia is not due to any local pathologic disturbance of the scalp, or more specifically, of the follicular apparatus. It is, therefore, not a true syphilide.

4. Clinical analogy affords the suggestion that it is due to endocrine dysfunction as a result of association and involvement of the autonomic nervous system.

5. Symptomatic alopecia representing a smaller group of the entire syndrome is a true syphilide, apparently caused by a perifollicular plasmoma.

REVIEWS

Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.

*Modern Clinical Syphilology**

IT IS obviously a matter of great difficulty—if, indeed, it can be said to be possible—to compress within the limits of a single volume of average size an adequate consideration of the intimate relation of syphilis to the practice of medicine and it is, therefore, inevitable, that any author contemplating a treatise upon syphilis must decide to whom his volume shall be addressed, the syphilographer or the practitioner at large.

Dr. Stokes has chosen the latter audience for his presentation and the resultant volume constitutes one of the most practical and usable works upon syphilis that it has been the privilege of the reviewer to examine. This book is of immediate practical interest and value to all who are interested in syphilis from whatever angle, to the laboratory worker who must have a working knowledge of clinical syphilology to apply intelligently his laboratory studies to the particular case, to the syphilographer because of its thorough survey and presentation of the modern knowledge of this subject, and most of all, perhaps, to the practitioner who essays to treat this omnigenous infection.

It is not to be denied, as has been said, that the development of both the Wassermann test and the arsenicals has had a tendency to develop a clan of pseudosyphilographers for whom the Wassermann test in the commercial laboratory makes the diagnosis while a few "shots" of "neo" suffice to treat the disease.

When such books as that of Dr. Stokes and his collaborators are available there is no excuse for pseudosyphilography.

After a brief discussion (26 pages) of the etiology, pathology, and immunology of syphilis, follow excellent and practical discussions of methods of clinical approach and physical examination excellently written and clearly illustrated.

Conveying a lesson, not always learned it would seem, there *follows* a clear discussion of the use and application of laboratory methods of examination which, it is emphasized, must be utilized and interpreted in conjunction with the other findings previously described.

Following this 138 pages are devoted to treatment: a discussion of the fundamental principles; a consideration of the uses of mercury, bismuth, the iodides and the arsenicals; and a clear, detailed and illustrated presentation of technic.

The remainder of the book is concerned with the diagnosis and treatment of syphilis in various stages and as affecting various organs and structures with final chapters on public health and miscellaneous aspects.

It is, of course, unnecessary to say that the book amply reflects an extensive and varied clinical experience.

It is throughout eminently practical, and specific. The illustrations are not only numerous and well reproduced but they *illustrate*. There are not only instructions as to what to do but clear directions, involving every step as to *how* to do it.

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*Modern Clinical Syphilology. By John H. Stokes, Professor of Dermatology and Syphilology, University of Pennsylvania, with the cooperation of P. A. O'Leary and W. H. Goeckermann of the Mayo Clinic, and L. W. Shaffer and C. J. White of the University of Pennsylvania. Pp. 1144. 865 illustrations and 866 figures. Cloth. Price $12.00 net. W. B. Saunders Co., Philadelphia.

NOTE: Insofar as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

An excellent feature are the numerous schematic comparisons, résumés, tabular summaries, case discussions, and aphoristic summaries—such as "the seven bads" in relation to arsphenamine tolerance; "the decalogue of dermatitis prevention" and the like which abound throughout the book.

This is a book which can be unreservedly commended and which can be purchased with assurance as to its immediate practical application to everyday problems.

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*Enzymes**

THE authors have endeavored to collect in concise form all the available information in regard to enzymes because, as they remark in their preface, "anyone who attempts a study of enzymes cannot fail to be struck by the vast accumulation of literature on the subject, while at the same time he will be confused by the many apparently contradictory results which have been published."

Modern studies of the chemistry of the living organisms both in health and disease, and the development of a broader and better understanding of the chemical activities of the bacterial causes of disease as well as what Wells has aptly termed the "chemical pathology" resulting have greatly extended this field of study.

The authors have endeavored to coordinate and correlate all the essential studies on this subject and, in so doing, have presented a most excellent monograph which should prove invaluable to all workers alike.

The clinician, the pathologist, and the laboratory worker alike will find this volume most useful, as—to quote again—"enzymes are formed by all living cells, whether the latter carry on all the functions of an organism, as in the case of unicellular forms of life, or are devoted only to specialized functions as in higher plants and animals."

"Life," say the authors, "is just one enzyme reaction after another," and, indeed, to a large extent a somewhat similar phrase could be applied to disease.

This monograph is not only exhaustive but excellently arranged and written.

It is divided into four major sections: I, Properties of Enzymes, 57 pages; II, Distribution of Enzymes, 60 pages; III, Methods for the Preparation and Study of Enzymes, 97 pages; IV, Practical Applications of Enzymes, 92 pages.

Methods are well described, generally those which the authors have found satisfactory in their own experience. A bibliography of 1323 references is appended evidencing the thoroughness with which the subject is reviewed.

The craftsmanship of the publishers is excellent, though one might wish for a smoother finish paper.

This work may be heartily recommended.

The Aspergilli†

THE authors remark very pertinently in the introduction to this book that the aspergilli —the "weeds" of the culture room—form a very considerable percentage of all the mold colonies encountered in the examination of soil, foodstuffs, and miscellaneous material.

In spite of their frequency these forms have been neglected and the literature concerned with their occurrence, characteristics, and relation to human and animal life is in a very chaotic and confused state.

The authors have been engaged in a study of this genus since 1904 and the present

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*Enzymes. By S. A. Waksman, Associate Professor of Soil Microbiology, Rutgers University and W. C. Davison, Associate Professor of Pediatrics, Johns Hopkins University. Pp. 364. Cloth. Price $5.50 net. Williams and Wilkins Co., Baltimore.

†The Aspergilli. By Charles Thom and Margaret B. Church, of the Microbiological Laboratory of The Bureau of Chemistry, Department of Agriculture. Pp. 272. 4 plates and 13 figures. Cloth. Price $5.00 net. Williams and Wilkins Co., Baltimore.

volume, embodying the results of long study of about 350 strains, is frankly biologic and primarily toxonomic in purpose.

It reflects in every page the extensive experience and the arduous labor of its authors and furnishes for the first time within the covers of one book a succinct but comprehensive discussion of the aspergilli which should prove of incalculable value.

After an historic discussion, followed by the description of a generic characterization, the morphologic classification is clearly discussed. Chapter III gives the basis of description and classification. Chapter IV is devoted to cultural methods.

In the following chapters are discussed: the physiologic and biochemic activities of aspergilli; the industrial significance of their enzymic and fermentative activities; and their relation to animal diseases.

The remaining 158 pages are devoted to group keys and a comprehensive description of those species which have been definitely classified. The final portion of the book presents a synoptic key, a list of accepted species and a list of 127 references.

The book thus presents, for the aspergilli, a source of reference similar to those available for the classification and identification of bacteria and should be at hand in every laboratory concerned with botanical or bacteriologic studies or food investigations.

The book is well bound and printed.

*Hydrogen-Ion Concentration**

THIS book, for the sake of continuity with its smaller predecessor, is called a second edition, but in view of the extensive as well as comprehensive expansion that this volume has undergone, it may well be regarded as a new book.

Neither Professor Michaelis nor his qualifications as an authority in this field require extended discussion.

So vast has been the amount of work done with regard to the significance of hydrogen-ion concentration in connection with biological sciences and so rapidly have technical procedures concerned with its determination undergone improvement that, in order to cover the field completely, a series of volumes is in contemplation, this being the first and concerned only with the theoretic physicochemical principles involved.

In this volume, because of the broadened realm of pure physical chemistry and the extraordinary growth of the multiplicity of applications of this branch to the other branches of science, the fundamental principles are placed upon a wider basis than before.

The volume deserves a wide circulation and should prove a valuable source of reference.

*Hydrogen-Ion Concentration. By L. Michaelis, Lecturer in Research Medicine, Johns Hopkins Hospital, translated into English by W. A. Perlzweig, Associate in Medicine, Johns Hopkins Hospital. Second Edition. Pp. 299. 32 figures. Cloth. Price $5.00 net. Williams & Wilkins Co., Baltimore.

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Editor-in-Chief: WARREN T. VAUGHAN, M.D.
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EDITORIALS

Liver Function Test

IN RECENT years much attention has been devoted to studies of liver function and to the development of clinically applicable means for measuring its efficiency or degree of impairment.

Probably no exaggeration is involved in considering the liver as one of the most important organs of the body; certainly there are few whose functions are more multiple.

Carlson[1] renewing our present knowledge of hepatic function cites the following:

1. Fairly conclusive experimental evidence is available that the liver is concerned with the coagulation of the blood in that fibrinogen is either produced in the liver or under its influence; certainly, at all events this is true of the regeneration of fibrinogen after hemorrhage.

It is also known that, under certain circumstances, the liver may produce substances capable of both acceleration and retardation of coagulation and

evidence exists indicative of the relation of the liver to various hemorrhagic diseases, particularly in infancy.

2. It is probable, though not as yet definitely proved, that the liver is the chief organ producing urea from the cleavage products of protein metabolism and from ammonia absorbed from the alimentary tract.

3. The intimate relation of the liver to carbohydrate metabolism, and its importance in relation to hypoglycemia, hyperglycemia, diabetes, and various forms of glycosuria is a problem not yet fully worked out but the importance of which is fully recognized and upon the study of the intricate mechanism of which a vast amount of work has been and is being done

4. That there is some relation, also, between the liver and fat metabolism is entirely probable, related, very possibly, to fat desaturation and oxidation.

5. As a source of bile, which seems both an excretion and secretion, the liver is of primary importance and the center of an intricate and far-reaching mechanism.

6. There is, finally, an interlocking relation between the nervous system and bile evacuation as well as liver function in general which, as Carlson remarks, forms an interesting and challenging chapter in the literature of liver function in health and disease.

In spite of the importance given to the liver even from the days of Hippocrates and the fact that it has long been regarded as exerting marked influence upon the functions of the body at large, its multitudinous functions are, apparently, only gradually coming to light.

Mann[2] has shown, in corroboration of others, that, following hepatectomy in dogs, marked changes occur in carbohydrate and protein metabolism and in the constituents of the bile with resultant disturbance of the bodily functions in general.

Health, in general, may be regarded as a condition characterized by, if not dependent upon, perfect performance of function. Disease, in turn may be broadly described as characterized or evidenced by disturbance of function. The essential value, therefore, of the development of means for the detection of functional inefficiency or the measurement of the degree of impairment is obvious and much attention has been devoted to the evolution of liver functional tests.

Because of the diversified nature of hepatic functions the problem is by no means an easy one and many methods have been proposed.

Rowntree, Marshall, and Chesney[3] have listed among the methods proposed:

1. Carbohydrate tests: inconstant and unreliable.

2. Nitrogenous studies of the urine: unreliable.

3. Tests for urobilin: useful as signifying liver damage but futile as a measure of the extent or kind of damage.

4. Determination of fibrinogen content of the blood because of the importance of the liver as a source of fibrinogen.

5. Estimation of blood lipose, because this is increased after liver damage experimentally produced.

6. The phenoltetrachlorphthalein test.

7. Tests for fibrinolytic ferment in the blood.

The two tests at present attracting particular attention are based upon: (a) the ability of the liver to remove certain substances from the blood with such accuracy that their impaired elimination may serve as a measure of hepatic injury; and (b) studies focused upon the most obvious hepatic secretion—and excretion—the bile.

In the first group the most work has been done with phenoltetrachlorphthalein, a dye substance first introduced by Rowntree, Hurwitz and Bloomfield[4] which is specifically excreted by the liver and which Whipple, Peighthal, and Clark[5] showed might be used as an index of liver damage.

Liver function may be measured, to some extent, by the intravenous injection of a known amount of this dye and the subsequent determination of the degree to which it has been removed from the blood after a definite interval.

Numerous reports have been made upon this procedure.

Friedenwald and Gautt,[6] using the duodenal tube method in 169 tests on 69 cases, report that there were but slight daily variations in the rate of excretion of the dye in the bile in the same individual; that starvation, age, or sex were without effect, and that the normal average appearance time of the dye in the bile was 13.8 minutes.

A slight prolongation was noted in pregnancy (8 cases); diabetes (2 cases) exerted no effect; in epilepsy (3 cases) a slight prolongation occurred (14.9 minutes), and also in malnutrition (2 cases) 15 minutes, while in catarrhal jaundice (2 cases) the excretion time was 10.5 minutes.

The following findings were encountered in the conditions listed: Addison's disease (1), 14 minutes; hyperpituitarism with epilepsy (1), 24 minutes; thyrotoxicosis (1), 17 minutes; secondary syphilis (2), 16 minutes; typhoid fever (2), 15.8 minutes; gallstone (40), 17 minutes; stone with jaundice (9), 28 minutes and (5) none in 60 minutes; hepatic carcinoma (1), 25 and 45 minutes; atrophic cirrhosis (2), 45 minutes and none in 60 minutes; arsphenamine jaundice (1), none in 2 hours; cancer of the pancreas (1), none in 2 hours; cardiac disease (1), myocarditis, 18 minutes; (1) chronic passive congestion, 13 minutes.

Ottenberg, Rosen and Goldsmith,[7] using the Rosenthal technic and determining the amount of dye remaining in the circulation after 15 and 60 minutes in 100 cases (in 14 cases after 1, 2, 3, 5 and 10 minutes), found it to be very rapidly removed, probably because 75 per cent of the total blood volume passes through the liver within two minutes and 96 per cent within five minutes.

They contend that this removal of the dye from the blood takes place whether the liver is able to excrete the dye or not and, therefore, that it is dependent, not upon excretion by the liver, but upon absorption from the blood. The amount of dye in the circulation at the end of 15 minutes indicates, according to these observers, the equilibrium between the presence of the dye in the blood and the absorptive capacity of the tissues, this point of

balance being, of course, lower when the liver is able to excrete some dye than in those instances in which the liver is obstructed. When the liver is badly damaged a very small part of the dye may be excreted by the kidneys.

As usually practiced, the determinations are made upon the blood; as modified by Piersol and Bockus,[8] the determinations are made upon the bile withdrawn by the duodenal tube, this being taken to indicate the amount of dye withdrawn from the blood in contrast to the amount retained by the blood and measured in the serum.

According to Hoxie,[9] this method is unreliable; Boardman and Schoonaker[10] find both methods of comparable value and prefer the duodenal tube method to avoid the thrombosis and induration consequent upon leakage of the dye into the tissues during or following its intravenous injection.

They further believe 50 mg. sufficient as a standard dose and that quantitative studies are impossible by the duodenal tube method because of the impossibility of collecting all the bile and that the test is, therefore, merely a qualitative test in which it suffices to measure the appearance time, more than eleven minutes indicating liver damage.

Pierson and Bockus, in a study of 67 cases, conclude that in obvious liver dysfunction the results of the test are clear and parallel the degree of pathology, but that some care is necessary in the interpretation of the test when only slight retention occurs.

They further conclude, from a study of arsphenamine-treated syphilis, that it is probable that some degree of liver dysfunction is present in all cases of syphilis undergoing arsphenamine treatment.

Greenbaum and Brown,[11] on the other hand, found no, or only slight, alteration in 70 per cent of cases with D.R.L. arsenicals, and believe that when toxic icterus occurs, there is apt to be a later permanent liver dysfunction not necessarily associated with apparent clinical disturbance.

Rosenfeld and Schneider[12] in a study of the test in pregnancy found no hepatic impairment in normal pregnancy; a definite indication of hepatic toxicity in the hypertension group, and a definite relation between the degree of dye retention and the severity of the symptoms in neurotic and toxic vomiting.

Bogen,[13] comparing a single sample taken one hour after injection with a set of permanent standards, accepts a retention of 3 per cent as normal, and interprets 4 to 8 per cent as indicative of partial hepatic insufficiency and over 8 per cent as significant of pronounced liver impairment.

Maurer and Gatewood,[14] working with rabbits, conclude that:

1. The dyes are removed from the blood stream as are other dyes and not by the liver alone.

2. The rate of removal is influenced to some extent by varying degrees of immunization.

3. The rate of removal from the blood is, apparently, not a true index of liver cell function impairment.

These investigators regard the intravenous method as not without danger in the presence of circulatory disturbances and prefer the duodenal technic as being the more logical.

They suggest that, for the purpose in view, the ideal dye must be, (a) nontoxic; (b) crystalline; (c) primarily removed solely by the liver; (d) not removed from the blood too rapidly in order to allow time for its estimation; and (e) ultimately removed solely by the liver parenchyma.

It is doubtful if such a substance is at present available.

Among those which have been proposed are Rose-Bengal by Kerr, Delpratt, Epstein and Dunievitz,[15] Azorubin S by Tada and Nakashima,[16] and Bromsulphalein by Rosenthal and White.[17]

All of these have their advantages and disadvantages, and all suffer from lack of definite proof that the liver only is concerned in their removal from the circulation.

The methods concerned with the excretion and retention of bile, therefore, are coming into somewhat general use and regarded as productive of information of definite clinical value.

Of these there are two in relatively common use, the qualitative detection of bile directly or indirectly by the Van den Bergh test and the quantitative measurement of the bilirubin content of the serum—the determination of the "icterus index"—by the various modifications of the method described by Muehlingracht.[18]

Each has its advocates and each has value.

As pointed out by Müller, Raydin and Raydin[19] these tests are particularly applicable to the detection of preicteroid states ("latent jaundice"), which may be compared to the increase of blood sugar before the occurrence of glycosuria.

The direct method of Van den Bergh measures the amount of bile in the blood serum; the indirect differentiates between the bilirubin remaining in the blood serum and that reentering from the bile.

The direct or immediate reaction is taken to indicate obstructive bilirubinemia; the indirect, as indicative of bilirubinemia of hemolytic origin in which, because of excessive hemolysis, bilirubin is formed in amounts too great for its rapid excretion by the polygonal hepatic cells.

Numerous investigators have reported upon these two tests which bid fair to assume a permanent place in the diagnostic armamentarium.

Bernheim,[20] for example, finds that clinical jaundice is invariably present when the icterus index is above 15 and absent below 15; the zone of latent jaundice lies, therefore, between 6 and 16.

In pernicious anemia the average icterus index was 10 in 10 cases; in secondary anemia 3.3 in 33 cases.

In malignancy a low reading indicates that anemia is not hemolytic in type. In 16 cases of duodenal ulcer the average reading was 9; in gastric ulcer it was normal. In malaria the index was 8; in typhoid fever 11.5, due posibly to gall bladder infection and focal necroses in the liver. In 41 car-

diac cases the index varied with the degree of compensation, cases with high readings proving fatal.

Similar reports are made by Maue[21] and many others.

There are numerous reports of investigations of Van den Bergh's test substantiating its clinical applicability so that it may be said with confidence that the methods concerned with the detection and measurement of bile pigments in the blood furnishes, at present, the method of choice in studies of hepatic functional impairment.

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—*R. A. K.*